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Severe Nutritional Macrocytic Anemia in Emotionally Disturbed Patients

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MOST PHYSICIANS are cognizant of the symptom complex of syncope due to sudden blood loss or vasomotor phenomena resulting in acute cerebral ischemia, but few are aware of the frequency of the abnormalities attributed to the nervous system in chronic anemia. The psychiatric or neurologic aberration may result not only from tissue oxygen depression related to the severity of the anemia, but also to altered cellular metabolism of the central nervous system seen in certain anemias of nutritional origin.[1–2] Conversely, emotionally disturbed patients may so alter their eating habits as to produce a deficiency state with resultant anemia.

In infants and children the most frequent deficiency state from which anemia may develop stems from inadequate intake of iron-containing foods. In adults iron-deficiency anemia occurs usually in women of the menstrual age group. This may be associated with abnormal blood loss in menses, pregnancy, or with dietary fads related to weight reduction. Chronic gastrointestinal bleeding is the most common cause of iron-deficiency anemia in men. Very rare are the nutritional macrocytic anemias produced by inadequate intake or absorption of folic-acid-containing foods. The altered hematologic picture resembles pernicious anemia, but many clinical features including glossitis, the lack of neurologic complications, and the usual presence of free hydrochloric acid in the stomach juices allow the differentiation to be made with ease in most instances. This communication reports observation on five emotionally disturbed patients who developed nutritional macrocytic anemia.

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CASE REPORTS

Case 1. D. P., a white female factory worker, aged 35 years, dated the onset of her illness two years previously. At that time full-mouth dental extractions were performed. Subsequent dentures which were ill-fitting proved unsatisfactory. The patient substituted milk for all solid food. She had eaten no meat for one year and for three months before admission had taken only milk and wine for nourishment. She had noted gradual onset of weakness; and for two months prior to admission had been forced to remain in bed because of exertional dyspnea, faintness, and palpitation. For two months she noted also painful sores in the mouth, puffiness of the face and ankles, and a thick, foul-smelling vaginal discharge with much vulvar irritation. Progressive mental depression and agitation developed in the six months before hospitalization.

Physical examination revealed a pale, bloated-appearing white woman who was markedly dyspneic on exertion. The temperature was 101° F., pulse, 100 per min, respirations, 20 per min, and weight, 135 lb. Pubic and axillary hair was sparce. Flame-shaped hemorrhages were seen in the retina on funduscopic

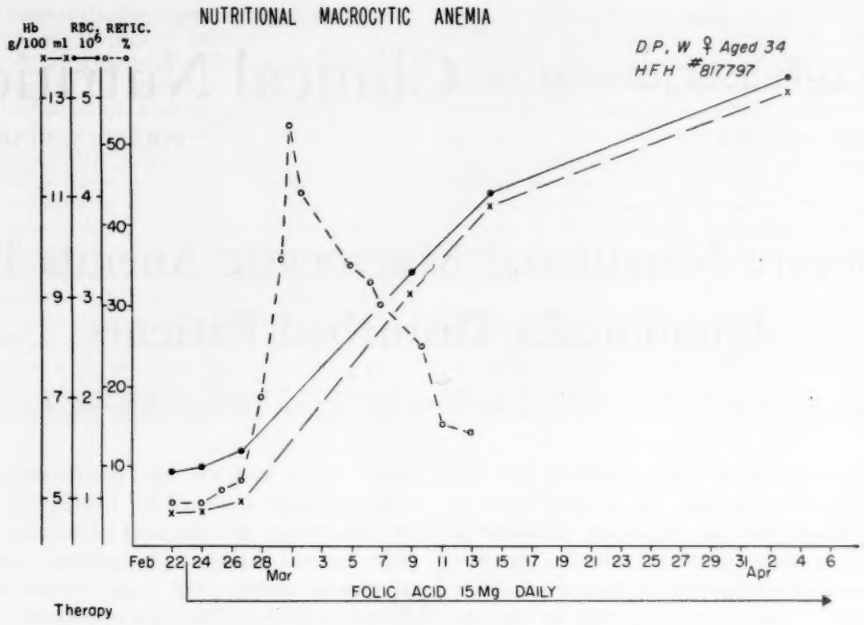
NUTRITIONAL MACROCYTIC ANEMIA
Hb g/100 ml
RBC, RETIC. 10⁶ %
D P, W ♀ Aged 34
H F H #817797
13 5 50
11 4 40
9 3 30
7 2 20
5 1 10
Feb 22 24 26 28 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 2 4 6
Mar
Apr
FOLIC ACID 15 Mg DAILY
Therapy

Fig. 1. Hematologic response to folic acid in nutritional macrocytic anemia.

examination. Many small, painful ulcers of the mucous membranes of the mouth and throat were evident. The teeth were absent. There was a generalized nontender lymph-adenopathy. About the ankles there were ecchymotic areas and a two-plus pitting edema. Vibratory and position senses were present. The vulva was red and swollen and a thick white vaginal discharge was noted.

On admission laboratory studies were reported as follows: hemoglobin, 5.7 g/ml; red blood count, 1.28 million/cu mm; white blood count, 3,750/cu mm. The blood smear demonstrated the erythrocytes to be macrocytic and several megaloblasts and many megalocytes were seen. The platelet count was 100,000/cu mm; reticulocyte count, 2.3 per cent; sedimentation rate, 0; hematocrit 9 mm. Urinalysis was normal. Blood nonprotein nitrogen, liver function tests, serum cholesterol levels, and van den Bergh tests were within normal limits. The blood albumin was 3.8 g/100 ml and globulin 1.6 g/100 ml. An oral glucose tolerance test was normal. Bone marrow smears revealed a hyperplastic picture with myeloid-erythroid ratio of 0.5:1. Megaloblasts and giant, developing hypersegmented neutrophils made up 64 per cent and 11.4 per cent respectively of the total blood cells. There was free hydrochloric acid in the stomach after histamine injection, and x-rays of the stomach, small bowel, and chest were within normal limits. Examination of the stool did not reveal blood or parasites. The blood vitamin A level was 25.6 mg/100 ml and vitamin C level was 3 mg/100 ml, both values are within normal limits. The Schilling test revealed 15.8 per cent of tagged-vitamin B_{12} in the urine.

The patient was treated with full diet and received folic acid 5 mg three times a day. Within four days the reticulocyte count was 19.8 per cent and reached a peak of 52.8 per cent in five days, slowly falling, thereafter, to 15.4 per cent on discharge three weeks after admission (Fig. 1).

She was seen one month later in the outpatient department and was completely asymptomatic. The hemoglobin now was 13.0/100 ml and erythrocytes 4.01 million/cu mm. During the interval of therapy marked improvement in the patient's irritability and confusion occurred.

Case 2. S. E., a white female, aged 38 years, sought medical care because of postprandial nausea and vomiting of one year's duration. During this interval her weight fell from 140 to 85 lb. Her diet at best consisted of one quart of milk with soggy crackers, one glass of orange juice a week, one half an egg a day. She would vomit a portion of this food each day. Her past history included a series of emotional problems, abnormal sexual practices, four marriages, alleged suicidal attempts, and a stint as an entertainer in night clubs of low repute.

On physical examination the patient was found to be agitated, emaciated, and she appeared much older than her stated age (Fig. 2). The mucous membranes of

Fig. 2. Case 2 (S. E.) demonstrating severe malnutrition with associated weight loss in an emotionally disturbed patient presenting a nutritional macrocytic anemia.

the mouth appeared mildly inflamed. The remainder of a general examination was negative but for vinyl implants in her breasts which presented an odd picture when viewed with her general cachexia. A psychiatric consultant classified her as demonstrating "psychophysiologic gastrointestinal reactions."

On admission her hematologic status was as follows: hemoglobin 9.8 g/100 ml, erythrocytes 2.80 million/cu mm, leukocytes 5,700 per cu mm. The erythrocytes were macrocytic on the blood smear. The reticulocytes were 1.6 per cent, platelets 198,000/cu mm. Bone narrow aspiration demonstrated slightly increased cellularity and 7.4 per cent of the nucleated cells were megaloblasts. Blood sugar, nonprotein nitrogen, and serum proteins were within normal limits. Free hydrochloric acid was found on gastric analysis. In the Schilling test the patient excreted 12.8 per cent of the tagged vitamin B_{12}. Roentgenograms of the gastrointestinal tract and bones were normal.

In addition to sedatives and antispasmodics, the patient received an emulsified regular hospital diet by intestinal intubation. The reticulocytes rose to a maximum of 26 per cent on this regime. Management of the patient was extremely difficult because of her emotional problem. After two weeks of tube feeding the patient was discharged. Continuation of this therapy at home proved unsuccessful because of the patient's uncooperative attitude.

One month after intermittent hyperalimentation, the hemoglobin had risen to 11.5 g/100 ml and erythrocytes to 3.25 million/cu mm. In spite of our energetic measures she failed to continue her medical treatment and was lost to follow-up.

Case 3. T. S., a 39-year-old housewife, was first seen in December, 1949 for the treatment of neurodermatitis of ten years' duration. Her extreme nervousness was attributed to an intolerable home situation. She complained of anorexia, ate no fresh fruits or vegetables and very little meat. For two years prior to admission, the patient complained of a painful tongue, an unsteady gait, generalized skin tenderness, and parasthesias from the hips down.

The patient was in a depressed state. Her weight was 136 lb. Her skin presented numerous dry scaly reddened lesions up to two inches in diameter, most numerous on the arms, legs, and chest. The tongue was red and somewhat atrophic. Mild generalized lymphadenopathy was present. Liver, spleen, and kidneys were not palpable. Neurologic examination revealed a slight foot-drop over the left common peroneal nerve distribution, decreased vibratory sense, and an absent position sense in the toes. The Romberg test was positive, and she was generally hyporeflexic.

The initial laboratory studies were reported as follows: hemoglobin 11.4 g/100 ml, erythrocytes 2.94 million/cu mm, white blood cells 3,600/cu mm. The red cells were macrocytic, and the platelets numbered 160,000 per cu mm. The reticulocyte count was 1.2 per cent; the sedimentation rate, 28 mm in one hour; hematocrit, 35 mm. Urinalysis, cephalin-cholesterol, bromsulphalein, van den Bergh tests, serum albumin and globulin concentrations, and fasting blood sugar were normal. A bone marrow aspiration revealed a moderately cellular marrow with a myeloid-erythroid ratio of 5.8:1. Giant developing neutrophils and megaloblasts were present. An increased number of megakaryoblasts with poor formation of platelets was reported. The gastric analysis showed free hydrochloric acid 30 minutes after histamine, and an x-ray of the stomach was normal.

The patient was placed on a full diet, thiamine hydrochloride, and 15 mg folic acid daily. In two weeks the tongue was normal. When discharged six weeks after admission the neurologic signs had disappeared. She continued on the same diet with a supplement of folic acid 5 mg daily. When last seen seven weeks after her admission she was asymptomatic. The hemoglobin was 13.2 g/100 ml and erythrocytes 4.16 million per cu mm. The nervous tension was much reduced.

Case 4. C. B., a white female aged 35 years, complained of weakness, exertional dyspnea, and ankle edema. The patient's husband, a druggist, volunteered the information that his wife was addicted to narcotics and that for the preceding two years her diet had been inadequate. Breakfast consisted of one slice of toast and coffee; lunch and dinner (which she only

ate about three times a week) a tuna fish or, rarely, a chicken sandwich. The weight loss was estimated to be about 20 lb in the last year.

Physical examination revealed a pale woman whose weight was 104 lb; tongue was normal except for a few small vesicles at the anterior edge; thyroid was slightly enlarged, and small nontender lymph nodes were palpable in the neck, axilla, and inguinal regions. The lungs and heart were normal except for a grade II systolic nonradiating apical murmur; spleen, kidneys, and liver were not palpable. A two-plus ankle edema was noted.

The initial hematologic data were as follows: hemoglobin 6.0 g/100 ml, red blood count 1.05 million/cu mm, white blood cells 4,650 per cu mm with a normal differential count. The erythrocytes were macrocytic, and many megalocytes were noted on the blood smear. The platelets numbered 218,000/cu mm, and the reticulocyte count was 2.8 per cent. The sedimentation rate was 20 mm in one hour and hematocrit 22 mm. Urinalysis and blood nonprotein nitrogen were normal. Liver function studies which included cephalin-cholesterol, thymol turbidity, and flocculation as well as bromsulphalein were normal. The blood cholesterol was 174 mg/100 ml; van den Bergh, direct 0.07 mg/100 ml, total 0.30 mg/100 ml. Serum albumin 3.9/100 ml and globulin 1.5 g/100 ml. An oral glucose tolerance curve was within normal limits, and a blood diastase was 60 per cent (normal = 50–150% Myer's method). Bone marrow aspiration revealed a hyperplastic marrow with a myeloid-erythroid ratio of 2:1. Numerous giant developing neutrophils and megaloblasts were seen. There was free acid in the stomach after histamine injection. X-ray study of the stomach and chest was normal. The stool fat was normal.

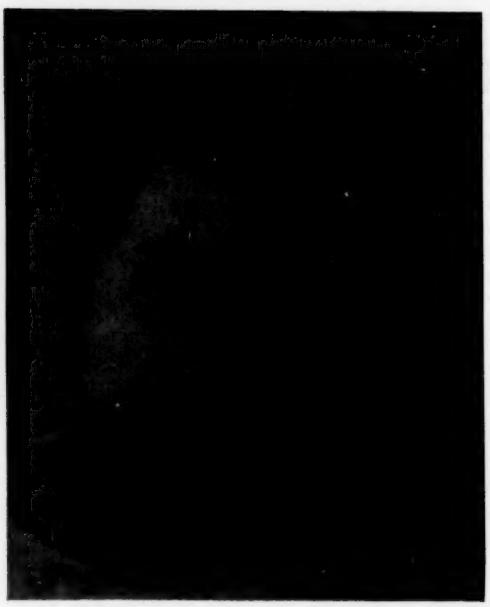

Fig. 3. Case 5 (H. B.) an emotionally depressed patient with nutritional macrocytic anemia. Puffiness of the face related to the anemia superficially obscures the recognition of severe underlying malnutrition.

The patient was placed on a full diet and given 15 μg of vitamin B_{12} intramuscularly daily for two weeks. Five days after therapy was instituted the reticulocyte count rose to 15.3 per cent and after ten days of treatment reached a maximum of 30 per cent. The hemoglobin on discharge from the hospital was 8.8 g/100 ml. Associated with the improvement in the blood picture the patient's agitation and anxiety improved rapidly.

She was followed as an outpatient for two months. During this interval she received 5 mg of folic acid three times a day. When last observed, she was asymptomatic. The hemoglobin reached 14.1 g/100 ml and erythrocytes 4.54 million/cu mm.

Case 5. H. B., a 28-year-old white housewife, admitted to the hospital with the main compiaint of weakness. She had been well until 18 months prior to admission at which time she had undergone great psychic trauma. Fuel oil which she had bought for a neighbor exploded, resulting in the death of five children. Many law suits followed. The patient became depressed and anorexic and for one year had eaten no meat or fruit. Her diet consisted mainly of milk and few vegetables. The patient's weight fell from 130 to 89 lb. She became progressively weaker and noted evening ankle edema. For four months before admission she had three to four soft brown stools daily and vomited when emotionally upset.

On physical examination she was found to be poorly nourished and pale, appearing much older than her stated age of 28 years (Fig. 3). Temperature 100.6° F., pulse 98 per min, weight 89 lb. Her skin was ashen, warm, and smooth. Funduscopic examination revealed numerous flame-shaped and round hemorrhages along the course of the larger vessels near the disc. Her tongue was smooth and painful and there were many small superficial ulcers of the oral mucosa. The lungs were normal, and a grade III systolic murmur was described in the apical area. Liver, spleen, and kidneys were not palpable. Neurologic examination was negative. A few small ecchymotic areas were noted over the lower extremities.

Laboratory studies performed on admission were as follows: hemoglobin 2.7 g/100 ml, erythrocytes 750,000 million/cu mm, and macrocytic erythrocytes were seen on the blood smear. Platelets numbered 45,000 per cu mm, and the reticulocyte count was 1.8 per cent. A sedimentation rate was 3 mm in one hour, and hematocrit 10 mm. Urinalysis, blood nonprotein nitrogen, van den Bergh, and cephalin-cholesterol,

bromsulphalein tests were normal. The serum albumin, globulin, and fasting blood sugar were normal. A bone marrow aspiration showed a myeloid erythroid ratio was 1.7:1, and the marrow was described as "so cellular the cells are arranged in sheets." There were 33 per cent giant developing neutrophils and 36 per cent megaloblasts. The gastric analysis revealed the presence of free acid on a fasting specimen, and an x-ray of the stomach and small bowel was normal. The stools were negative for ova, parasites, and blood.

The patient received 500 cc whole blood transfusion on admission. Thereafter, she was placed on a full diet, vitamins A and B, Brewer's yeast, refined liver extract 1 cc intramuscularly daily, and folic acid 10 mg three times a day. On this therapy her reticulocyte count rose in five days to 17.6 per cent and in two weeks to a peak of 23.6 per cent. On discharge her hemoglobin was 11.9 g/100 ml, and a bone marrow aspiration showed only slight increase in cellularity with a myeloid erythroid ratio of 2.2:1. Normoblastic regeneration was good, but there were a few scattered megaloblasts.

The patient was discharged on Brewer's yeast three times a day and on a full diet. When seen one month later she was asymptomatic, and her hemoglobin was 13.6 g/100 ml and erythrocytes 4.25 million/cu mm. Coincident with her hematologic improvement she became more alert with marked relief of her depression.

DISCUSSION

These cases have in common: young emotionally disturbed females, inadequate food intake, glossitis, severe macrocytic anemia, megaloblastic bone marrow, free hydrochloric acid in stomach juices without evidence of liver disease or gastrointestinal absorptive defect. Two patients responded adequately to folic acid, one to hyperalimentation, one to vitamin B_{12}, and one to refined liver extract (Table I). It is of interest that in each instance all patients were lost to follow-up after a two-month period because of lack of cooperation. During the period of therapy the mental status improved remarkably only to relapse at a later date. Fragmentary information in three patients indicated a return of both nervous symptoms and the hematologic disturbances.

This group of patients undoubtedly represented states of multiple deficiencies indeed, Case 3 (T. S.) presented characteristic signs and symptoms of polyneuritis, presumably thiamine depletion.

The B-complex vitamins are essential for the maintenance of a normal central nervous system. For example, riboflavin deficiency produces no known hematologic or neurologic disease in man. Thiamine alterations may be associated with polyneuritis, nervousness, apathy, depressions, and parasthesias without significant changes of the blood.[3] Lack of nicotinic acid is often followed by symptoms and signs of "dementia, dermatitis, and diarrhea." The failure of absorption of one μg of vitamin B_{12} daily by the gastrointestinal tracts results in a systemic combined degeneration of the cord and transient emotional instability.

Nutritional macrocytic anemia, nontropical and tropical sprue, and macrocytic anemia of pregnancy respond specifically to folic acid in a manner to suggest the anemia results from

TABLE I

Hematologic Results in the Treatment of Nutritional Macrocytic Anemia

Case	Pre-treatment			Therapy	Post-treatment			
	Hb* g/100 ml	RBC million	Megaloblasts bone marrow, %		Maximum reticulocytes, %	Hb, g/100 ml	RBC million	Period of therapy
1. D. P.	5.7	1.28	64.0	15 mg folic acid q.d.	52.8	13.0	4.01	6 wk
2. S. E.	9.8	2.80	7.4	Tube feeding	26.0	11.5	3.25	1 mo
3. T. S.	11.4	2.94	4.8	15 mg folic acid q.d.	10.2	13.2	4.16	7 wk
4. C. B.	6.0	1.05	38.1	Vit. B_{12} 15 μg q.d. I.M. for 2 wks, then 15 mg folic acid q.d.	30.0	14.1	4.54	2 mo
5. H. B.	2.7	0.75	36.0	500 cc whole blood; refined liver extract 1 cc I.M. q.d. for 2 wk, then 10 mg folic acid q.d.	23.6	13.6	4.25	2 mo

* Hb = hemoglobin; RBC = red blood cell count (millions/cu mm); q.d. = daily; I.M. = intramuscularly.

inadequate intake or absorption of this essential vitamin. The pathophysiology of the defect in these diseases is still not clear. Folic acid deficiency has been produced in dogs with resultant bone marrow hypoplasia, hypochromic anemia with mild microcytosis and glossitis. Megaloblastic anemia can be produced in monkeys by feeding milk diets deficient in ascorbic acid.[4] Folic acid deficiency has been produced in pigs. The hematologic manifestations observed in these animals were (a) severe macrocytic anemia, (b) leukopenia, (c) thrombocytopenia, and (d) hyperplastic bone marrow with increase in immature nucleated red cells which resemble megaloblasts observed in pernicious anemia.[5] On the basis of microbiologic data, the theory has been proposed that folic acid and vitamin B_{12} act as coenzymes at different stages of nucleoprotein synthesis. An interruption of this process results in a megaloblastic picture in the bone marrow followed by a macrocytic anemia. (The mechanism of this disturbance has been discussed in detail by Mueller and Will.[6])

CONCLUSIONS

Emotional instability may be associated with severe nutritional deficiency with resultant macrocytic anemia. The anemia and altered cellular metabolism may augment the nervous symptoms.

The macrocytic anemia responds promptly to folic acid, vitamin B_{12}, refined liver extract, and hyperalimentation. The combination of folic acid and adequate supportive psychosomatic therapy offers the most favorable treatment.

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The Effect of Dietary Protein, Fat, and Choline upon the Serum Lipids and Lipoproteins of the Rat

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IN MAMMALS, the function of all cells is influenced by the composition of the internal environment of the body. As Claude Bernard noted, numerous mechanisms exist for maintaining this internal environment constant in order that cellular physiologic processes may proceed optimally. When marked changes in the internal environment do occur, as a result of malnutrition or breakdown in homeostatic mechanisms, aberrations in cellular function occur with ultimate disease and often death of the organism.

In malnutrition, changes occur as a result of failure of the external environment to provide essential nutrients for the replenishment of the internal environment and eventually of the cells themselves. The relationship between the length and extent of dietary deficiency of an essential nutrient and the changes in concentration of that nutrient in plasma and tissue cells of the mammalian organism has been studied for a number of essential nutrients.[1] That relationships of this type should exist is not surprising for compounds which cannot be synthesized by the body. It is somewhat more remarkable to recall that the composition of the diet may also influence the concentration of compounds which can be synthesized by the body such as glucose, fat, and cholesterol. Interest in this area of diet therapy has been intensified recently by the realization that the composition of the internal environment with regard to lipids and lipoproteins is one factor which influences the rate of atherogenesis. A study of the mechanisms by which diet influences the composition of the serum with regard to lipids and lipoproteins should cast additional light upon the homeostatic mechanisms by which the organism maintains its *milieu interne*.

In this paper are reported some experiments which delineate the role of the lipotropic factors, choline and methionine, in the regulation of the serum lipids and lipoproteins in the rat. This study actually began 18 years ago when I (the senior author of this paper) was a graduate student in the laboratory of Wendell Griffith at St. Louis University. Dr. Griffith had just described the syndrome of hemorrhagic renal degeneration as a manifestation of choline deficiency in the weanling rat,[2] and suggested to me that I undertake a study of the overall lipid metabolism of the young choline-deficient rat with particular reference to the blood lipids. I very willingly accepted this problem, developed the methods, and made some preliminary observations upon the effect of various dietary constituents, particularly choline, upon the liver, blood, and carcass lipids of weanling rats. Some of these data, obtained in 1940, depicting the relationship between dietary choline and the serum lipids,

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TABLE I

Effect of Choline upon the Serum Lipids of Weanling Rats*

Experiment	Number of rats	Basal diet	Choline %	Renal lesions %	Liver fat %	Serum lipids, *mg/100 ml*			
						Total lipid	Phospholipid	Cholesterol	
								Total	Free
1	24	AC51†	0.00	93	29.1	464	164	86	29
2	16	AC51	0.02	69	30.8	353	98	76	20
3	18	AC51	0.08	0	18.5	496	151	99	33
4	25	AC51	0.15	0	5.8	523	170	106	38

* R. E. Olson and W. H. Griffith (St. Louis University, 1940).

† Basal diet, AC51: casein 18, sucrose 48.6, cystine 0.3, salts 4.0, calcium carbonate 1.0, agar 2.0, brewers yeast 6.0, lard 20.0, natola 0.1.

are shown in Table I. The most severely deprived animals, 93 per cent of whom showed renal hemorrhage, did not show the most marked hypolipemia. This anomalous result, we found later, was due to the severe inanition which these animals suffered as a result of their renal lesions. Unfortunately this work was interrupted by the exigencies of the war and was not resumed by me until two years ago.

During the past several years there has been a great interest in the dietary factors which influence serum lipids in man. This has developed because of epidemiologic evidence suggesting[3] that the intake of dietary fat correlates with average serum cholesterol level and with mortality from coronary heart disease in various populations. For many years it has been suspected that a relationship existed between hypercholesterolemia and coronary artery disease in man[4] but it has become increasingly apparent that other factors (both local arterial and humoral) play a role as well.[5] It is possible, however, that lowering the serum cholesterol in susceptible humans may be an important preventive measure and hence any dietary or other technic which appears to be practicable in this regard should be thoroughly investigated. It was with this in mind that, about two years ago, we sought to repeat at the University of Pittsburgh some of the experiments which had been carried out in Dr. Griffith's laboratory many years before. In searching the literature we discovered a paper by Ridout, Patterson, Lucas, and Best[6] in which they showed that the feeding of cholesterol to rats over a ten-week period did not elevate the ester cholesterol level of the serum unless choline was present in the diet. This observation gave us additional encouragement to carry out the studies which are presented below.

MATERIALS AND METHODS

The use of weanling animals was avoided because of the proneness with which they develop renal hemorrhage on low-choline diets. Young Sprague-Dawley male rats of the Holtzman strain weighing about 120 g

TABLE II

Design of Experimental Diets

Constituent	Composition of diets, %			
	A	B	C	D
Casein	—	—	18.0	18.0
Soy Protein*	18.0	18.0	—	—
Fat†	5.9	39.9	5.9	39.9
Sucrose	69.0	35.0	69.3	35.3
Salts (H. M. W.)	4.0	4.0	4.0	4.0
Cod Liver Oil	2.0	2.0	2.0	2.0
Cystine	0.5	0.5	0.2	0.2
α-Tocopherol‡	0.1	0.1	0.1	0.1
B-Complex§	0.5	0.5	0.5	0.5
Choline	±0.3	±0.3	±0.3	±0.3

* Soy protein was supplied by Drackett Products Co., Cincinnati 32, Ohio.

† Fat (lard, corn oil, or butterfat as indicated in protocols).

‡ 10 mg/cc α-tocopherol/Wesson oil.

§ Vitamins were added in a sucrose vehicle in the following amounts expressed as milligrams per kilogram of diet: thiamine 5, riboflavin 8, pyridoxine 5, calcium pantothenate 20, niacin 40, folic acid 1.5, biotin 0.05, menadione 0.08, and vitamin B_{12} 0.1.

were used in these experiments. They were divided into groups of 12 animals each and fed respectively the 16 experimental diets summarized in Table II and the stock ration, Purina Chow. The experimental diets shown represent the permutations of low/high methionine, low/high choline, low/high fat, and saturated/unsaturated fat at the high level of 40 per cent. The range of dietary content of methionine was 0.16 per cent (soy protein) to 0.54 per cent (casein); the range for choline was 0–0.3 per cent; and the range for fat was 6–40 per cent by weight. The total organic sulfur of these rations was kept constant at 0.2 per cent by varying the content of cystine. Lard, butterfat, and corn oil were used as sources of fat.

Each group was fed the experimental ration for two weeks and sacrificed by needle aortotomy under nembutal anesthesia. In this manner 4 to 6 ml of blood were obtained from each animal and blood samples from four animals pooled for determination of the serum lipids and lipoproteins. The livers were removed, weighed, and quick-frozen. Total serum and liver lipid was determined by the micro-oxidative method of Bloor,[7] total serum cholesterol by the method of Abell *et al.*,[8] and serum phospholipid on chloroform-methanol extracts by the method of Youngberg.[9] Serum lipoproteins of both the high-density (which flotate in saline of density 1.21 and comprise most of the α-lipoprotein of the electrophoretogram of serum) and the low-density type (which flotate in saline of density of 1.063 and comprise most of the β-lipoprotein of the electrophoretogram of serum) were determined by the analytical ultracentrifugal technics of Gofman and associates.[10]

TABLE III

Serum Lipids in Man and Rat

	Man	Rat
Lipids		
Total	550	250
Cholesterol	220	90
Phospholipid	250	140
Phospholipid: Cholesterol ratio	1.1	1.5
Lipoproteins		
High-density (α) (Flotate 1.21)	150 ($-S_8$)	125 ($-S_8$)
Low-density (β) (Flotate 1.06)		
S_f 0–12	250	35
S_f 12–20	35	0
S_f 20–100	75	40
S_f 100–400	50	15

RESULTS

Young adult rats fed a stock ration (Purina Chow) show relatively low levels of serum lipids[11] when compared to man.[12] They also demonstrate a considerably different profile of serum lipoproteins than man. As noted by Lewis, Green, and Page[13] rats have much lower levels of low-density β-lipoproteins than man. The high-density (α)-lipoprotein concentrations are comparable but the flotation rate in the rat is slightly faster than the corresponding α-lipoprotein in man. Our studies completely confirm these observations. Typical values for the serum lipids and lipoproteins of rat and human serum are

TABLE IV

Effect of Choline and Fat upon the Serum Lipids and Lipoproteins of Rats Fed Low-Choline, Low-Methionine (Soy Protein) Diets

Diet			Liver lipid	Serum lipids, *mg/100 ml*			Serum lipoproteins, *mg/100 ml*					
								Low density (β)				
Group	Lard %	Choline %	Total %	Cholesterol	Phospholipid	Total	High density, (α)-S_8	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	Total
Chow	—	—	4.1	90	139	252	125	38	0	37	14	89
A-1	6	0.0	30.1	49	95	150	90	2	0	2	0	4
A-2	6	0.3	5.9	87	188	341	150	28	0	62	74	164
B-3	40	0.0	35.6	50	126	166	147	0	0	0	0	0
B-4	40	0.3	6.2	93	184	341	195	37	0	38	13	88

presented in Table III. It is noteworthy that the rat has no β-lipoprotein of the class S_f 12–20.

The effects of specific dietary variables upon the serum lipids and lipoproteins and upon liver fat in the rat are shown in Tables IV through VII. Each group of rats is designated by a letter and number, the letter indicating which of the basic diets listed in Table I was fed. Additional identifying data relative to fat and choline intake are given for each group. Animals fed the soy protein rations devoid of choline and low in methionine developed markedly fatty livers and marked generalized hypolipemia with decrease in serum cholesterol, phospholipid, and total lipid and a virtual disappearance of low-density lipoproteins. In group A-1, which received 6 per cent lard, the high-density lipoproteins declined 30 per cent, whereas in group B-3, receiving 40 per cent lard, there was no change in the high-density lipoprotein fraction. The addition of choline to these rations prevented the fatty liver, the hypolipemia, and the hypo-betalipoproteinemia. The choline-supplemented animals tended to have somewhat higher total lipid, phospholipid, and high-density lipoprotein levels than even the chow-fed controls. In general there was a moderately good correlation between the serum phospholipid values and the high-density α-lipoprotein concentrations.

When diets containing casein instead of soy protein were fed, the hypolipemic effects were minimal even in the absence of choline as shown in Table V. It is assumed that the extra methionine in this ration (C-5) contributed its labile methyl group for choline synthesis. This assumption is supported by the finding of much less fatty infiltration of the liver in rats on this ration than on diet A-1. Low-density β-lipoprotein concentrations in the serum of animals fed diet C-5 were lower than chow-fed animals and lower than corresponding control rats given choline (diet C-6). Increasing the fat concentration from 6 to 40 per cent markedly increased the fatty infiltration of the liver in rats given the casein-containing diets without an appreciable change in the lipids or serum lipoproteins as noted in group D-7. This suggests that this effect of labile methyl concentration in the diet upon serum lipids is not solely a function of the degree of fattiness of the liver although it is true that the most marked hypolipemias were seen in association with severely fatty livers.

In the next series of experiments reported in Tables VI and VII, corn oil and butterfat were compared with lard at the level of 40 per cent in the diet. All fats gave essentially identical results as regards degree of fatty infiltration attained in the two week period, degree of hypolipemia and hypolipoproteinemia in the absence of choline on both soy and casein diets. Neither in the presence nor the absence of choline was the hypolipemic effect of unsaturated dietary fat reported in man[14–16] observed in the rat.

RELEVANCE TO MAN

The relevance of these findings in the rat to man is an important question. As is well known, one cannot transpose data from

TABLE V

Effect of Choline and Fat upon the Serum Lipids and Lipoproteins of Rats Fed Diets Adequate in Methionine (Casein) but Low in Choline

Diet			Liver lipid	Serum lipids, *mg/100 ml*			Serum lipoproteins, *mg/100 ml*					
								Low density (β)				
Group	Lard %	Choline %	Total %	Cholesterol	Phospholipid	Total	High density, (α)-S_8	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	Total
Chow	—	—	4.1	90	139	251	125	38	0	37	14	89
C-5	6	0.0	7.4	75	163	264	105	15	0	5	28	48
C-6	6	0.3	3.6	84	159	313	128	17	0	45	60	112
D-7	40	0.0	22.9	88	165	250	120	22	0	18	15	65
D-8	40	0.3	5.8	90	166	291	158	38	0	28	8	74

TABLE VI

Effect of Type of Fat (Corn Oil vs. Butterfat) on the Serum Lipids and Lipoproteins of Rats Fed High-Fat, Low-Methionine (Soy Protein) Diets with and without Choline

Diet			Liver lipid	Serum lipids, *mg/100 ml*			Serum lipoproteins, *mg/100 ml*					
								Low density (β)				
Nr.	Lard %	Choline %	Total %	Cholesterol	Phosph. lipid	Total	High density, (α)-S_6	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	Total
Chow	—	—	4.1	90	139	251	125	38	0	37	14	89
B-9	Bu	0.0	29.1	56	102	178	119	3	0	1	0	4
B-10	Bu	0.3	8.5	99	171	364	181	23	0	31	9	63
B-11	Co	0.0	26.5	59	104	193	142	2	0	0	0	2
B-12	Co	0.3	8.9	106	177	366	210	28	0	35	14	77

TABLE VII

Effect of Type of Fat (Corn Oil vs. Butterfat) on the Serum Lipids and Lipoproteins of Rats Fed High-Fat Diets Adequate in Methionine (Casein) with and without Choline

Diet			Liver lipid	Serum lipids, *mg/100 ml*			Serum lipoproteins, *mg/100 ml*					
								Low density (β)				
Nr.	Lard %	Choline %	Total %	Cholesterol	Phosph. lipid	Total	High density, (α)-S_6	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	Total
Chow	—	—	4.1	90	139	251	125	38	0	37	14	89
D-13	Bu	0.0	20.8	91	134	286	103	19	0	13	8	40
D-14	Bu	0.3	6.3	101	148	365	145	30	3	41	8	82
D-15	Co	0.0	17.8	90	142	264	120	30	0	10	1	41
D-16	Co	0.3	5.8	104	171	344	155	42	0	28	2	72

species to species without pertinent evidence. In another primate, the Cebus monkey, Mann *et al.*[17] found that hyperlipemia from cholesterol feeding was not obtained unless choline was added to the diet. The rice diet introduced by Kempner[18] for the treatment of hypertension is one of the most potent hypocholesterolemic rations known for man. It contains 25 g of protein, 5 g of fat, and carbohydrate in quantity sufficient to maintain caloric balance. Methionine appears to be one of the limiting amino acids. Although the hypolipemic effect of this ration has been attributed to its low fat content, the decreases in serum cholesterol are out of proportion to what has been observed on diets as low in fat but replete in protein.[19] Preliminary data obtained on our own metabolic unit with vegetable diets including the rice diet low in total protein but moderate in fat (80 to 90 g daily) suggest that protein may be an important determinant of serum lipid levels in man as well as the rat.[20] Present evidence would support the view that methionine is the crucial amino acid although others may also be involved. Vegetable diets containing reduced amounts of poor quality protein produce kwashiorkor in human infants which is characterized by fatty liver and hypocholesterolemia.[21] Even the epidemiologic evidence adduced by Keys to implicate dietary fat as partially causative of coronary artery disease can be equally well interpreted to implicate animal protein. In Figure 1 is shown the data cited by Keys[3] relating per cent of calories derived from dietary fat to mortality from degenerative heart disease in males 55 to 59 years. In Figure 2 are shown the same data on mortality from degenerative heart disease plotted against dietary intake of animal protein in grams per day. As one can see, the curves are practically identical. This, of course, does not prove that the intake of dietary fat has no bearing on the pathogenesis of coronary artery disease any more than it proves that the intake of animal protein is

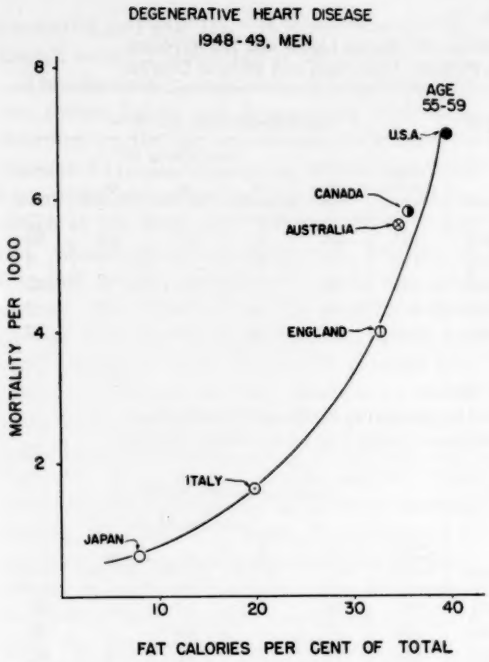

Fig. 1. Association of fat intake with mortality from degenerative heart disease. (Redrawn from Keys, A.[3])

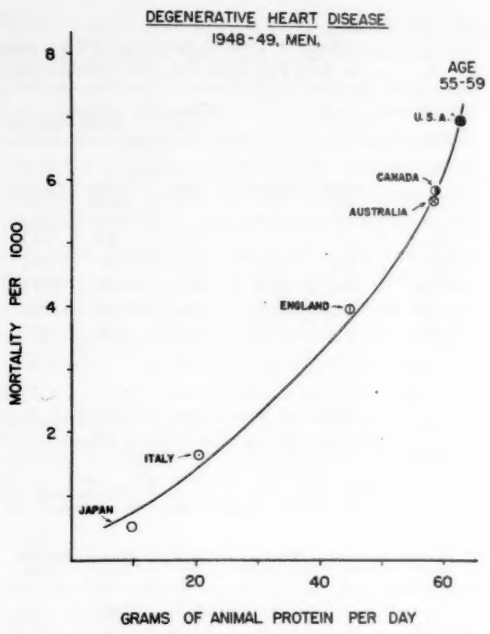

Fig. 2. Association of animal protein intake with mortality from degenerative heart disease. (Mortality data from Keys, A.[3]; data on animal protein intake from Woytinsky, W. S., and Woytinsky, E. S.[25])

the critical factor. Epidemiologic data are never proof of causation; by the same token a role for animal protein has not been excluded by the available data.

DISCUSSION

Choline deficiency in the young adult rat causes a marked alteration in fat transport with a decrease in the level of cholesterol and phospholipid, a reduction in the high-density α-lipoproteins and virtual disappearance of the low-density β-lipoproteins. Wilgram, Lewis, and Blumenstein[22]* described similar changes in serum cholesterol and lipoproteins in choline deficient rats. The decrease in low-density β-lipoproteins observed by them was not as marked as observed by us, possibly because their dietary protein, consisting of peanut meal, casein and soy protein supplied more methionine than did our basal ration.

The substitution of casein for soy protein entirely prevented the hypocholesterolemia and minimized the hypolipoproteinemia seen on the soy protein diets even though it did not, at all levels of dietary fat, completely prevent fatty infiltration of the liver. This effect is presumably due to the *labile methyl group* of methionine and is opposite to the effect observed by Fillios and Mann[23] in rats fed α-protein diets containing 5 per cent cholesterol. These workers observed that the hypercholesterolemia obtained by feeding rats a diet containing soy protein 20 per cent, corn oil 15 per cent, cholesterol 5 per cent, and choline 0.2 per cent could be reduced by supplementation with 0.6 per cent DL-methionine.

Since choline, but not cystine was present in the basal diet, this diet was adequate in labile methyl but inadequate in total organic sulfur. The effect of the added methionine therefore was not due to the labile methyl group but was due to the additional sulfur supplied. Subsequently Portman and Mann[24]

* Published while this work was in progress.

found that taurine would substitute for cystine or methionine in their experiments in lowering serum cholesterol, presumably by increasing taurocholate output in the bile. This variable was avoided in our experiments by keeping the total organic sulfur constant.

It would appear that for the rat the withdrawal of choline and other labile methyl donors from the diet is a more effective means of lowering the serum cholesterol and β-lipoprotein concentrations than varying the kind and amount of dietary fat. The hypocholesterolemic effect of choline deficiency was similar on all levels of dietary lard even though the caloric content of the rations varied from 4.3 (low fat) to 5.9 (high fat) cal/g and the protein intake varied respectively from 2.2 to 1.8 g per rat per day. In these experiments, varying the degree of unsaturation of the dietary fat at 40 per cent by weight (63 per cent of calories) from that obtained with corn oil (56 per cent linoleic acid) to that obtained with butterfat (4 per cent linoleic acid) had no effect upon the level of serum lipids or lipoproteins in the rat with or without added choline. The significant changes in serum lipids in man caused by alteration of the degree of unsaturation of dietary fat serves to re-emphasize the species differences in fat transport. The precise mechanism of the low-choline effect upon the serum lipids of the rat is not fully known and is being studied in our laboratory by isotopic methods.

It is not to be inferred from these data that low-protein diets are recommended for the control of hypercholesterolemia or of atherosclerosis in man. The value of an adequate protein intake replete with the essential amino acids in the maintenance of health in adults is proved. It is probable nevertheless, that the protein intake of adult Americans generally is above the minimum required for health. Populations subsisting on minimum protein intakes in the far and middle East and in Africa, as well as alcoholics in our country, tend to have more liver disease and less atherosclerosis than better nourished populations. The weight of the evidence, both experimental and clinical, would support the view that the liver elaborates the β-lipoproteins which promote atherogenesis, and that this process is sensitive to alteration of dietary protein, lipotropic factors, and fat. The extent to which diet therapy can be used in the prevention of atherosclerosis in man is a subject for continuing research.

SUMMARY

Young adult rats develop a marked hypocholesterolemia, hypolipemia, and hypobetalipoproteinemia when fed soy protein diets low in methionine and choline. These effects are prevented by the addition of 0.3 per cent choline to the diet and are partially prevented by casein, presumably because of its increased methionine content. The level and type of dietary fat (ranging from 6 to 42 per cent and including butterfat, corn oil, and lard) did not modify this effect of choline upon serum lipids. A review of the available clinical, experimental, and epidemiologic data on man would suggest that the lipotropic factors as reflected principally by animal protein intake may play a role in controlling serum lipids in man as well as the rat.

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The Role of Nutritional Factors in the Antibody Responses of the Anamnestic Process

A. E. Axelrod, PH.D.*

Previous studies in this laboratory have demonstrated the significant role of the vitamins in the processes of antibody production. In these investigations, the effects of *specific*, individual vitamin deficiencies upon antibody production in the albino rat were determined. In early experiments with human erythrocytes as the antigen it was shown that antibody synthesis was markedly impaired in pantothenic acid, pyridoxine, and pteroylglutamic acid deficiencies.[1] Lesser degrees of inhibition were noted in other vitamin deficiency states; in vitamin D and vitamin B_{12} deficiencies no effects were apparent. It was also demonstrated repeatedly that simple inanition failed to modify the antibody response.

These experiments were subsequently extended to include purified, alum-precipitated, diphtheria toxoid as the antigen.[2] Again, the marked impairment of antibody response in various vitamin deficiency states was evident. With the exception of the deleterious action of the vitamin D deficiency, the trends observed with diphtheria toxoid were similar to those previously noted when red blood cells served as the antigen. Here too, simple inanition was without effect.

The experiments described in this report were designed to investigate the role of nutritional factors in the various phases of the anamnestic process.

METHODS AND MATERIALS

Male, weanling, albino rats of the Holzman strain were employed in all of the vitamin experiments. Rats weighing 90 g were utilized in the tryptophan study. Each animal was housed in an individual, wide-meshed cage. The individual vitamin deficiencies were produced by feeding the purified diets previously described.[2] The control rats in each instance received the basal, vitamin-deficient diet supplemented with the crystalline vitamin in question. For the production of a tryptophan deficiency, the casein of the control diet was replaced by acid-hydrolyzed casein and the diet supplemented with 0.6 per cent of DL-methionine. For the tryptophan controls this basal diet was further supplemented with 0.2 per cent of DL-tryptophan. All rats were fed *ad libitum* and were utilized in groups of 20 for each experimental procedure.

The intraperitoneal injection of 0.15 ml of purified, alum-precipitated, diphtheria toxoid (Lederle) served as the antigenic stimulus throughout these studies. Blood was obtained from nembutalized animals by cardiac puncture and serum antibody titers determined on the heat-inactivated sera by a sensitive, serial-dilution, hemagglutination technic which is dependent upon the agglutination of tannic acid-treated sheep erythrocytes coated with

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diphtheria toxoid.[3,4] The content of serum antibody is expressed as the reciprocal of the highest serum dilution capable of a positive hemagglutination reaction and, therefore, varies *directly* with the reciprocal titer.

EXPERIMENTAL PROCEDURE

A typical anamnestic response in normal rats to diphtheria toxoid is shown in Figure 1.

In all of the vitamin studies weanling rats were fed the experimental diets for four weeks prior to the primary injection of antigen. In the tryptophan experiment, the animals were maintained for two weeks on their respective diets prior to antigenic stimulation. The serum antibody titers determined three weeks after the primary injection constitutes the *primary response* and this three-week interval is designated as the *primary phase* of the anamnestic process. Lessening the time of the primary phase diminishes the magnitude of the secondary response. Immediately following the withdrawal of blood for the determination

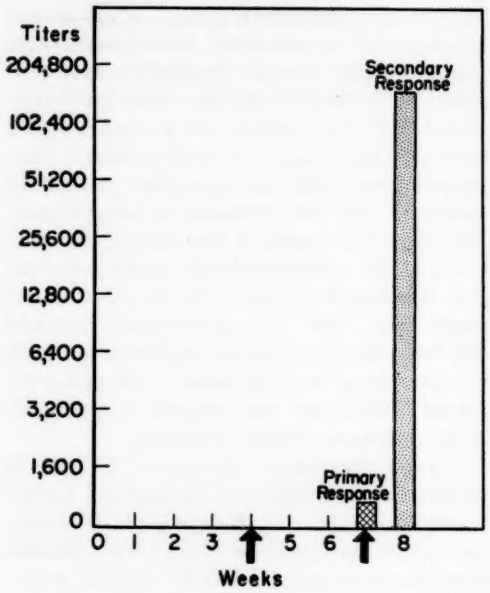

Fig. 1. The anamnestic response in control rats. Weanling rats were placed on experiment at zero weeks and the primary and secondary injections of diphtheria toxoid given at the times indicated by the arrows. Figures on the ordinate represent reciprocal titers.

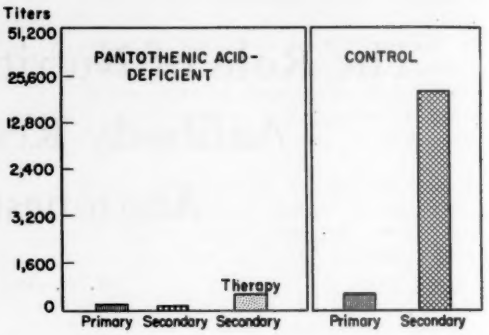

Fig. 2. Anamnestic responses to diphtheria toxoid in pantothenic acid-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 1 mg of pantothenic acid daily by intraperitoneal injection only during the secondary phase.

of the primary response, the animals were given a second or booster injection of antigen. The ensuing week is referred to as the *secondary phase* of the anamnestic process and the antibody titer at the end of this week is the *secondary response*. It is seen in Figure 1 that the booster injection produces a very sharp increase in antibody titer. The relatively low

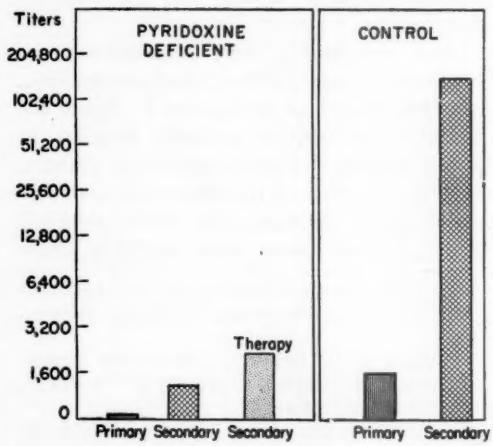

Fig. 3. Anamnestic responses to diphtheria toxoid in pyridoxine-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 500 mcg of pyridoxine daily by intraperitoneal injection *only* during the secondary phase.

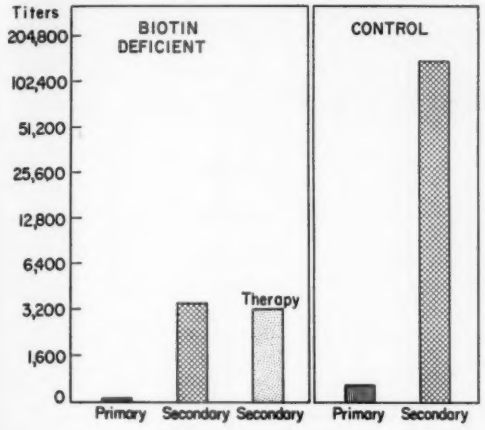

Fig. 4. Anamnestic responses to diphtheria toxoid in biotin-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 10 γ of biotin daily by intraperitoneal injection *only* during the secondary phase.

primary response is maintained for several weeks in the absence of a secondary antigenic stimulus.

As will be illustrated later in this report, deficiencies of a number of nutritional factors during the *entire* experimental period inhibit both the primary and the secondary responses. The present experiments can be conveniently divided into two phases, each purporting to answer one of the following questions: (1) Can nutritional therapy *only* during the secondary phase elicit a normal secondary response in animals which have been nutritionally deficient during the period prior to the booster injection? and (2) Conversely, can a nutritional deficiency *only* during the secondary phase inhibit a secondary response in animals fed adequate diets prior to the booster injection?

Phase 1

In this phase of the investigations, deficiencies of pantothenic acid, pyridoxine, biotin, and tryptophan were induced and primary and secondary responses to diphtheria toxoid obtained as described above. Each experiment included a group of deficient animals which received intensive nutritional therapy during the one-week secondary phase as indicated in Figures 2, 3, 4, and 5. Typical signs of deficiency, in particular growth inhibition, were evident at the time of the secondary injection

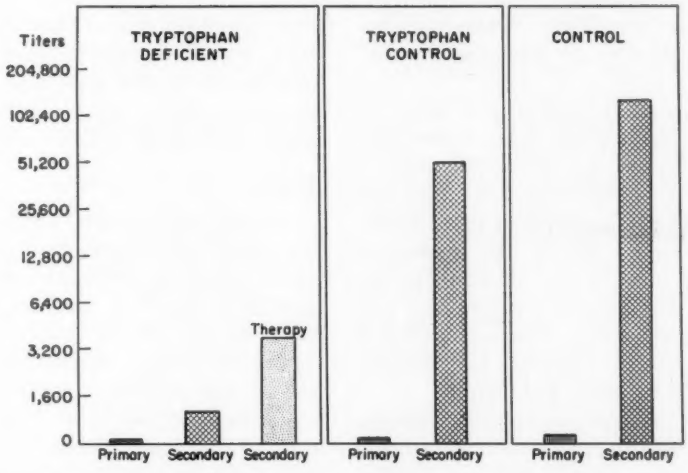

Fig. 5. Anamnestic responses to diphtheria toxoid in tryptophan-deficient, tryptophan-control, and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the tryptophan control diet *only* during the secondary phase.

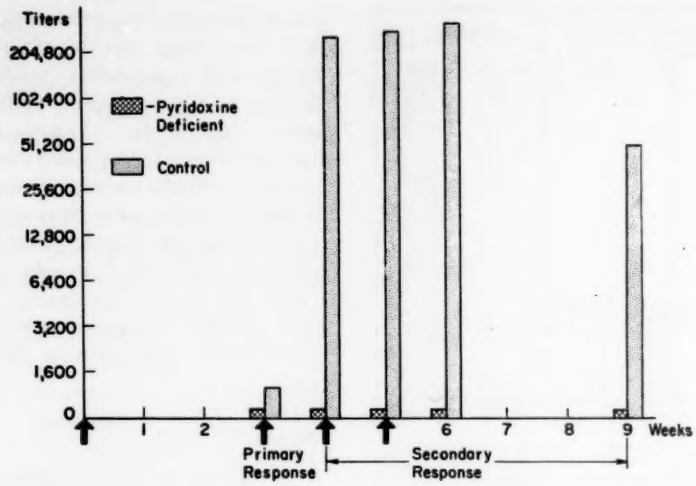

Fig. 6. Effect of repeated antigenic stimuli upon antibody response in pyridoxine-deficient and control rats. Injections of diphtheria toxoid given at times indicated by the arrows. Animals were fed the pyridoxine-deficient or the control diet during the entire course of the study, and were on experiment for four weeks prior to the first toxoid injection. Figures on the ordinate represent reciprocal titers.

and the usual immediate marked responses to the nutritional therapies were noted. Thus, weight gains of 30 to 40 g were observed during the week following therapy. In the tryptophan study an additional control group receiving the usual control diet (which contains unhydrolyzed casein as the source of protein) was utilized.

The results given in Figures 2, 3, 4, and 5 depict the inhibitory effects of the nutritional deficiency states upon the anamnestic process. The impairment of the secondary responses under these circumstances is most pronounced. Nutritional therapy during the secondary phase has very little, if any, effect upon the antibody response. In no case was an adequate anamnestic response seen in these supplemented animals despite their immediate and marked growth responses to nutritional therapy.

A subsequent experiment (Figure 6) demonstrated that repeated injections of diphtheria toxoid following the original booster injection failed to produce an antibody response in pyridoxine-deficient rats. Not shown in this figure are the observations that: (1) Pyridoxine therapy instituted concomitantly with the booster series failed to elicit an immediate anamnestic response but, after two weeks, did produce a mild increase in circulating antibodies comparable to the usual primary response. A true anamnestic response was seen in about 50 per cent of these animals one week later. (2) Pyridoxine-deficient rats not receiving any booster injections were unable to produce antibody despite pyridoxine therapy begun three weeks after the primary stimulus.

Phase 2

In these experiments, a study was made of the effect upon antibody production of an acute pyridoxine deficiency induced during the secondary phase of the anamnestic process with the aid of the pyridoxine antagonist, desoxypyridoxine. During the primary phase the animals received a control diet furnishing ten instead of the usual 50 μg of pyridoxine daily. This lower amount of pyridoxine permitted good growth (25 g per week) and antibody production and was utilized in order to facilitate the action of the desoxypyridoxine. Administration of the desoxypyridoxine to rats fed a "pyridoxine-free" diet produced an

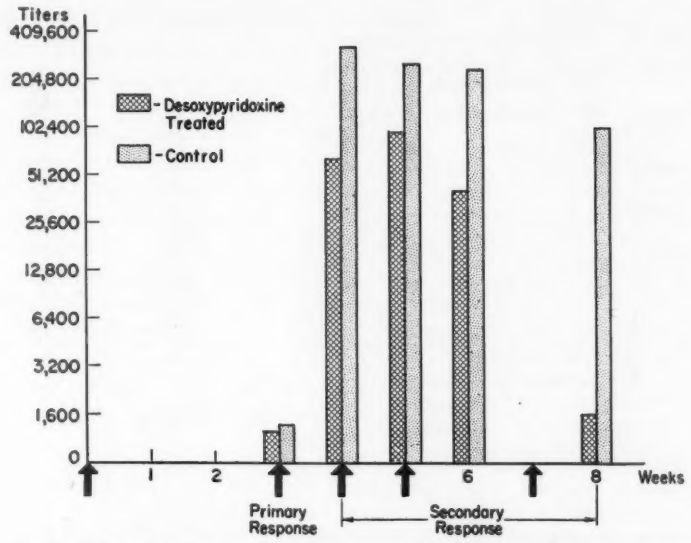

Fig. 7. Effect of desoxypyridoxine-treatment during the secondary phase upon antibody response. Injections of diphtheria toxoid were given at the times indicated by the arrows. *All* animals were fed a control diet furnishing 10 μg of pyridoxine daily for four weeks prior to the first injection of the toxoid and during the subsequent three-week primary phase. Following the determination of the primary response, the animals were divided into two groups; one continued to receive the control regimen while the other was fed the basal "pyridoxine-free" diet and given daily intraperitoneal injections of 5 mg of desoxypyridoxine per rat. Figures on the ordinate represent reciprocal titers.

immediate weight decline and the appearance of typical pyridoxine deficiency symptoms within a week. During a five-week period of this acute pyridoxine deficiency the animals lost an average of 80 g.

The results of the first experiment in this series are shown in Figure 7. In the control group the typical pronounced secondary response was observed in every animal one week following the initial booster injection and was maintained at a high level during the succeeding four weeks. In contrast, the secondary response of the desoxypyridoxine-treated group was significantly diminished. Thus, 40 per cent of these rats failed to show a true secondary response one week following the first booster injection and a marked fall in antibody titer occurred at the fifth week despite continued antigenic stimulation.

The second experiment (Fig. 8) was designed to obtain further information on the time course of the secondary response to a single booster injection and thus to delineate more sharply the effects of a pyridoxine deficiency upon this response. The plan of this experiment was similar to the preceding one with the exception that only a *single* booster injection was given and antibody titers were determined three, six, and nine days later. In order to ascertain the specificity of the desoxypyridoxine, one group was supplemented with pyridoxine during the period of desoxypyridoxine administration in the secondary phase.

In the control rats, the appearance of circulating antibodies was noted three days after the booster injection and the titers increased to a maximum value at nine days. In agreement with the preceding experiment, the antibody responses were diminished in the pyridoxine-deficient group. This inhibition was detectable three days after the booster injection. Antibody titers similar to those of the control group were observed in the desoxypyridoxine-treated rats supplemented with

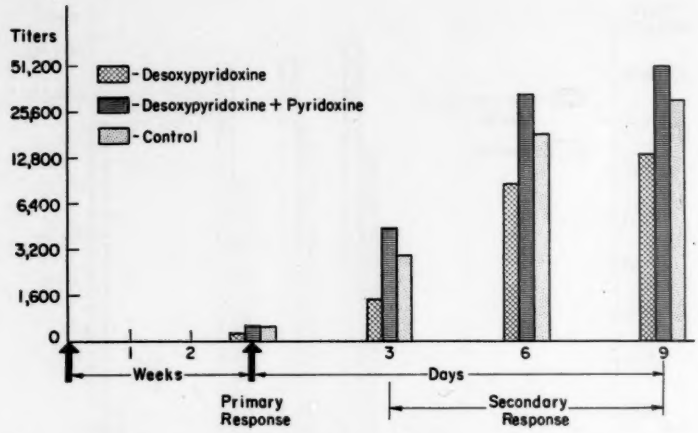

Fig. 8. Effect of desoxypyridoxine-treatment during the secondary phase upon antibody response. Experimental details were essentially those described in Figure 7 with the exception that 5 mg of desoxypyridoxine were given twice daily. An additional desoxypyridoxine-treated group received 1 mg of pyridoxine daily by intraperitoneal injection.

pyridoxine. All other symptoms attributable to desoxypyridoxine were relieved by pyridoxine.

DISCUSSION

The precise mechanism of the anamnestic process is not known with certainty. A number of theories, the discussion of which is outside the scope of this paper have been proposed and have met with varying degrees of acceptance. Whatever the mechanism, it is apparent that events, e.g. template or adaptive enzyme formation, transpire during the primary phase which are triggered by the secondary stimulus to initiate or, more likely accelerate, the processes of antibody synthesis. What then is the significance of the present findings when viewed within this framework of the anamnestic process?

It had been previously established in this laboratory that the primary antibody responses of the albino rat to the antigenic stimulus of diphtheria toxoid were markedly impaired in deficiencies of pyridoxine, pantothenic acid and biotin. The present study clearly demonstrates that the inhibitory effects of these deficiencies upon the secondary responses to this antigen are even more pronounced. Similar results were noted in a tryptophan deficiency. In these cases, the administration of the missing nutritional factor resulted in immediate weight gains but had very little effect upon antibody production. It seems, therefore, that the elaboration of the primary events of the anamnestic process does not occur in the absence of these nutritional factors and, accordingly, it is not surprising that the secondary stimuli are ineffective under these circumstances. The failure of nutritional therapy during the secondary phase to promote antibody production further emphasizes the absolute need for adequate nutrition *during the primary phase* in order to ensure the attainment of the complete anamnestic process. Continued nutritional therapy eventually permits the initiation of a typical primary response.

The second series of experiments demonstrated that a pyridoxine deficiency induced by administration of desoxypyridoxine during the secondary phase could significantly inhibit the secondary response. This inhibition was apparent three days after the booster injection. It should be stressed that the events of the primary phase proceeded in these experiments while the animals were in a state of good nutrition. Thus, a pyridoxine deficiency in the secondary phase can inhibit the sequence of events normally resulting from the trigger-

ing action of the secondary antigenic stimulus. This harmful effect of the deficiency is evident despite the existence of a normal functional mechanism elaborated during the primary phase. The delayed inhibitory action of the pyridoxine antagonist can be attributed to the partial stimulation of the secondary response occurring prior to the full impact of the deficiency state. This is evident from the failure to maintain the elevated anamnestic titers of circulating antibody during the prolonged, acute pyridoxine deficiency.

The above results are in conformity with those obtained by Stoerk[5] and can most likely be ascribed to the deleterious effects of the acute pyridoxine deficiency upon the lymphoid elements (cf. Mushett *et al.*[6] and Stoerk).[7] Stavitsky[8] has shown that the popliteal lymph node and the spleen are definitely involved in the enhanced response to a second injection of diphtheria toxoid in the rabbit. In the same species, Dixon[9] has demonstrated that the secondary response to a variety of particulate antigens is radiosensitive and he suggests that the radiosensitive process involves the preparation of particulate antigens by the host for antibody synthesis. Numerous experiments involving organ extirpation, cell transfer and *in vitro* culture have implicated the lymphoid apparatus in the processes of antibody formation.[8,10–13]

CONCLUSION

The successful attainment and maintenance of a satisfactory anamnestic response to diphtheria toxoid in the albino rat requires a state of adequate nutrition during both the primary and secondary phases of this process.

ACKNOWLEDGMENTS

The author wishes to acknowledge the excellent technical assistance rendered by Daina Macins and Sarah Hopper. He also wishes to express his indebtedness to the Lederle Laboratories, Inc. for generous gifts of alum-precipitated diphtheria toxoid and fluid diphtheria toxoid.

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Nutritional Status of Selected Adolescent Children.*

II. VITAMIN A NUTRITION ASSESSED BY DIETARY INTAKE AND SERUM LEVELS. BIOMICROSCOPIC AND GROSS OBSERVATIONS

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IN 1952 a nutritional status study was conducted with Washington-born and -reared, 15 and 16-year-old children. These subjects were drawn from two areas in the State of Washington, Snohomish and Yakima Counties. Both boys and girls participated in the study, 63 boys and 62 girls from Snohomish County; 61 boys and 62 girls from Yakima County. A detailed description of the selection of areas and subjects was reported in a previous paper in this JOURNAL.[1]

The purpose of the present paper is to discuss the vitamin A nutrition of the selected adolescent children. Indices which contribute to the evaluation are: (1) a seven-day dietary record, (2) vitamin A and carotene blood serum levels, (3) biomicroscopic examination of the bulbar conjunctiva and epithelium of the upper arm, (4) gross observations—colored photography of the eye, and physical examination of the eye and skin of the upper arm.

EXPERIMENTAL PROCEDURE

Seven-Day Dietary Record: The subject recorded semi-quantitatively his or her food intake for seven consecutive days.[1] The information obtained was used to calculate the daily mean intake, and standard error of the mean for dietary vitamin A. The results were compared with the National Research Council Recommended Allowances.[2]

Vitamin A and Carotene Blood Serum Levels: The method of Bessey *et al.*[3] was used to determine the serum vitamin A and carotene blood levels. The determinations were made on fasting blood samples.

Biomicroscopic Examination: The bulbar conjunctiva and epithelium of the upper arm were examined with a Bausch and Lomb Poser Slit Lamp. All biomicroscopic observations were made by the same operator.

To observe xerosis of the bulbar conjunctiva, the conjunctiva of each eye was divided into temporal and nasal portions, and each portion was redivided into thirds: limbic, middle, and canthic. Each third was subdivided into upper, median, and lower areas. Each area was observed, and a qualitative value allotted: transparent; slight, some or marked translucency; or opaque. Since it is difficult to observe accurately the canthic third, this section was not evaluated quantitatively. The qualitative descriptions for each of the remaining 24 areas were arbitrarily assigned quantitative values as follows: transparent, 1; slight translucency, 2; some translucency, 3; marked translucency, 4; and opaqueness, 5. Therefore, the more severe the lesion, the higher the value becomes. A mean value for the 24 areas, 12 on each eye, was obtained for each subject.

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The posterolateral aspect of the left upper arm was examined with the biomicroscope for signs of xerosis (dryness), thickening of the epithelium, keratinization, and hyperkeratosis with spines. A qualitative value of "normal," slight, some, marked or very marked was assigned to each of these lesions. Each qualitative description was arbitrarily quantified by giving "normal" the value of 1; slight, 2; some, 3; marked, 4; and very marked, 5. The mean value was obtained for each subject.

Colored Photography: Both eyes of each subject were photographed using a Leica camera with a ground glass copy attachment using Kodachrome Type A film. The camera was $9^3/_4$ in. from the conjunctiva of the subject. Each slide was classified as good, fair or poor, denoting the degree of xerosis of the bulbar conjunctiva. The observations were made by the individual who evaluated the biomicroscopic findings. For the best results, the examiner viewed the slides at a distance of approximately 9 feet from a 40 in. × 54 in. screen. The qualitative results were arbitrarily assigned quantitative values of good, 1; fair, 2; poor, 3. The mean value for each subject was calculated.

Physical Examination: The eyes and epithelium of the upper part of the arm were examined during the physical examination of each subject. One physician made the examinations in Snohomish County; a second physician in Yakima County. The conjunctiva was examined for signs of thickening and Bitot's spots; the skin of the upper arm for xerosis, folliculosis and hyperkeratosis (elbows). These observations were quantified as absent, 1; mild, 2; moderate, 3; severe, 4. The mean values were obtained for each lesion for each subject.

Statistical: Mean values were calculated for Snohomish County boys, Snohomish County girls, Yakima County boys and Yakima County girls for: dietary intake of vitamin A; serum vitamin A and serum carotene; biomicroscopic, kodachrome and physical values for xerosis of the bulbar conjunctiva and epithelium of the upper arm. Simple and pooled simple correlations were calculated in an effort to establish relationships between dietary intake, various blood constituents and subclinical signs associated with a vitamin A deficiency.

RESULTS AND DISCUSSION

Seven-Day Dietary Record: Table I shows the mean daily intake of vitamin A, without supplements. Only 16 subjects had vitamin A supplementation, and 15 of these already had an adequate vitamin A intake, without supplementation.

No significant difference was found between the intakes of vitamin A of the 15- and 16-year olds, either boys or girls.[1] The intake of the boys was higher than that of the girls and this difference was highly significant. Area differences corrected for sex and area differences within sexes were not significant.

The vitamin A intake (arithmetic mean) of the Washington boys was much lower than that found for similar groups in Iowa,[4] Maine,[5] and nonrheumatic subjects in Utah.[6] However, the intake compares favorably with the intake of the subjects studied by Tucker *et al.*[5] in New York. The Washington boys had a higher vitamin A intake than the boys in Rhode Island,[5] Montana,[7] and Idaho.[8] The

TABLE I

Mean Daily Intake of Vitamin A (IU)

	Snohomish County		Yakima County	
	Boys (63)	Girls (62)	Boys (61)	Girls (62)
Mean and standard error of mean	8263 ± 952.02	5740 ± 500.77	7619 ± 587.43	5922 ± 680.88
Geometric mean	6939	4929	6673	4649
Geometric mean logarithm and standard error	3.8413 ± 0.0295	3.6927 ± 0.0290	3.8246 ± 0.0276	3.6674 ± 0.0358

Geometric mean and the standard error of its logarithm were used to decrease skewness in these populations.

TABLE II

Mean Serum Levels of Vitamin A and Carotene

Serum constituent	Snohomish County		Yakima County	
	Boys (63)	Girls (62)	Boys (61)	Girls (62)
Vitamin A (μg/100 ml)	46.4 ± 1.05*	44.7 ± 0.89	45.7 ± 1.27	43.6 ± 1.72
Carotene (μg/100 ml)	117.1 ± 6.47	113.8 ± 5.10	105.9 ± 5.07	107.6 ± 4.37

* Mean and standard error of mean.

arithmetic mean vitamin A intake of the Washington girls was lower than the intake of the girls in similar age groups in Iowa,[4] Maine and New York;[5] similar to the intake in Rhode Island,[5] the nonrheumatic subjects in Utah[6] and Idaho;[8] and higher than the intake in Montana.[7]

The mean daily vitamin A intake was compared with the National Research Council Recommended Allowances.[2] The Snohomish boys consumed 165.3 per cent of the recommended allowance for vitamin A, the Snohomish girls 114.6 per cent, Yakima boys 152.3 per cent, and Yakima girls 110.8 per cent. Only 37.9 per cent of the Washington boys consumed less than the recommended allowances compared with 52.4 per cent of the white boys of the same age group in Tennessee[9] and 61.0 per cent of the boys in New York City.[10] The percentage of Washington girls consuming less than the recommended allowances was similar to that found by Youmans *et al.*[9] in Tennessee and Wiehl[10] in New York City—57.7, 68.4 and 63.0 per cent, respectively.

Serum Vitamin A and Serum Carotene: Table II shows the mean blood serum levels for vitamin A and carotene.

No significant difference was found between the serum vitamin A or carotene blood serum levels of the 15- and 16-year-olds, either boys or girls; or between the serum levels of the boys and girls of the same age group. Area differences corrected for sex and area differences within sexes were not significant for either vitamin A or carotene.

The mean serum vitamin A values for both the Washington boys and girls were higher than those reported for adolescents in other areas: Michigan,[11] Idaho,[12] Northeast Region,[13] Vermont,[14] Oregon,[15] and Utah,[16] but lower than those reported in Montana.[17]

The mean serum carotene values for Washington subjects were lower than the values reported by Beach *et al.*[11] in Michigan, Bring, Warnick and Wood[12] in Idaho, Wilcox *et al.*[16] in Utah and Odland and Ostle[17] in Montana. Krause[14] in Vermont reports a value of 101 μg per 100 ml of carotene in the serum for subjects with folliculosis and 116 μg per 100 ml for subjects without folliculosis. Carotene levels for adolescents in Maine[13] and Oregon[15] were lower than those found in Washington. The serum levels for the boys in New York State and Rhode Island[13] were almost the same as those found in this study but the values for the girls in New York were higher than the values for the Washington girls. The serum levels for those in Rhode Island were lower.

Serum vitamin A levels less than 20 μg/100 ml are considered by Bessey and Lowry[18] to indicate a "poor" level of nutrition. None of the participants in this study were in this classification. Bessey and Lowry classified serum carotene levels as "poor" when they fell below 75 μg/100 ml. Seventeen per cent of the Snohomish boys and 16 per cent of the Snohomish girls were in this classification; 23 per cent of the Yakima boys and 18 per cent of the Yakima girls could be classified as "poor." The mean serum vitamin A value indicated that these subjects could be classified as in a "good" state of nutrition, whereas the mean serum carotene value would classify them as "fair."

BIOMICROSCOPIC OBSERVATIONS

Prior to the concept of the deficiency states proposed by Kruse[19] in 1942, little recognition had been given to the subclinical signs of malnutrition associated with avitaminosis. According to Kruse[20] each avitaminosis could be depicted as showing a tissue specificity. The

relative thickness of the bulbar conjunctiva could be influenced by vitamin A nutrition. The conjunctival thickness was ascertained by studying light transmission through it with the biomicroscope. Other investigators, notably Berliner,[21] Darby,[22] and Kodicek and Yudkin[23] have questioned the theory advanced by Kruse that a particular tissue lesion is pathognomonic for a specific deficiency.

The biomicroscope has been used as an aid in studying the nutritional status of adolescents in surveys conducted by Mack and Urbach[24] and Wiehl and Kruse.[25] Following the theory presented by Kruse[20] that subclinical signs of avitaminosis A may be observed in specific tissues, the bulbar conjunctiva of the eyes and the epithelium of the arm of the subjects in this study were observed with the biomicroscope.

Xerosis of the Bulbar Conjunctiva: The mean quantitative values obtained for xerosis of the bulbar conjunctiva showed that only very slight differences existed among the four groups. Snohomish boys had a mean score of 2.5, Snohomish girls 2.4, Yakima boys 2.8, and Yakima girls 2.6. The mean for all boys was 2.6 and for all girls 2.5. Interpreted according to the method of evaluation adopted for this study, these values indicate that the state of xerosis of the conjunctiva of all four groups could be described as being between "slight" and "some" translucency. In turn, this finding could indicate that some avitaminosis A was present among these subjects.

Epithelium of the Upper Arm: The mean quantitative scores for the biomicroscopic observations of the epithelium of the upper arm are shown in Table III. The mean scores indicate that a very slight difference existed among the groups in this respect. Based on the scores obtained, if the specificity of this lesion is accepted as being indicative of avitaminosis A, then it would appear that there is very slight evidence of a deficiency of vitamin A among these adolescent children studied.

GROSS OBSERVATIONS

Colored Photography: Colored photographs have been used to record the subclinical observations noted in adolescent subjects in surveys by Beach *et al.*[11] and Browe and Pierce.[26]

The mean quantitative values for xerosis of the bulbar conjunctiva, as judged from kodachrome slides, were 2.4 for both the Snohomish boys and girls, 2.5 for Yakima boys and 2.6 for Yakima girls. Based on the criteria used in this study, xerosis for these subjects could be described as "fair" to "poor."

The colored photographs taken for the study were very clear. The details of the conjunctiva of the eye were readily observed. Under conditions of exceedingly good photography, it is thought that greater use of photographs might be made to supplement the observations seen with the biomicroscope. These photographs comprise a permanent record which can be reviewed. Merrow *et al.*[27] state that colored slides are a useful tool in the evaluation of subclinical signs of malnutrition.

Physical Observations: No subclinical symptoms associated with vitamin A malnutrition were noted by the examining physician in Snohomish County, for either the conjunctiva of the eye or the skin of the upper arm. No evidence of either thickening or Bitot's spots was noted in the examination of the conjunctiva of the subjects in Yakima County.

The mean quantitative score for the physical

TABLE III

Mean Quantitative Scores for Biomicroscopic Observations of the Epithelium of the Upper Arm

Lesion	Snohomish		Yakima		All boys (124)	All girls (124)
	Boys (63)	Girls (62)	Boys (61)	Girls (62)		
Xerosis	1.5	1.7	1.7	1.8	1.6	1.8
Thickening	1.3	1.3	1.1	1.2	1.2	1.2
Keratinization	1.4	1.7	1.4	1.4	1.4	1.6
Hyperkeratosis with spines	1.3	1.2	1.1	1.2	1.2	1.2
Mean	1.4	1.5	1.3	1.4	1.4	1.4

TABLE IV
Simple Correlation Coefficients

	County	Sex	Serum			Xerosis conjunctiva		Keratinization of upper arm		Vitamin A intake without supplements
			Vitamin A	Carotene	Vitamin C	Biomicroscopic	Kodachrome	Biomicroscopic	Physical	
Vitamin A intake with supplements	Snohomish	Boys	0.145	0.245	0.494†	−0.060	−0.224	−0.129	—	—
		Girls	0.135	0.401†	0.294*	−0.069	0.053	−0.014	—	—
	Yakima	Boys	0.072	0.378†	0.317*	0.015	−0.064	−0.039	−0.122	—
		Girls	−0.075	0.099	0.190	−0.091	0.006	−0.044	0.045	—
Vitamin A intake from plant sources	Snohomish	Boys	0.098	0.347†	0.599†	−0.139	−0.112	−0.238	—	—
		Girls	0.169	0.286*	0.313*	−0.140	0.185	−0.010	—	—
	Yakima	Boys	0.177	0.377†	0.267*	−0.016	−0.118	−0.029	−0.219	—
		Girls	−0.163	0.379†	0.198	0.042	−0.035	−0.073	−0.181	—
Serum vitamin A	Snohomish	Boys	—	0.409†	0.317*	−0.073	−0.282*	0.120	—	—
		Girls	—	0.471†	0.428†	0.035	0.021	−0.119	—	—
	Yakima	Boys	—	0.361†	0.111	−0.010	0.054	−0.276*	−0.111	—
		Girls	—	0.142	0.418†	0.150	0.018	0.196	0.050	—
Serum carotene	Snohomish	Boys	—	—	0.431†	−0.232	−0.234	0.008	—	—
		Girls	—	—	0.392†	−0.122	−0.234	0.155	—	—
	Yakima	Boys	—	—	0.351†	−0.046	−0.056	−0.013	−0.230	—
		Girls	—	—	0.494†	0.248	−0.022	0.125	−0.140	—
Serum vitamin C	Snohomish	Boys	—	—	—	−0.119	−0.130	−0.226	—	—
		Girls	—	—	—	−0.153	0.131	−0.080	—	—
	Yakima	Boys	—	—	—	−0.288*	−0.053	0.007	−0.038	—
		Girls	—	—	—	−0.046	−0.087	0.029	−0.062	—
Xerosis conjunctiva (Biomicroscopic)	Snohomish	Boys	—	—	—	—	0.189	0.232	—	−0.097
		Girls	—	—	—	—	−0.015	0.023	—	−0.045
	Yakima	Boys	—	—	—	—	0.221	0.078	0.151	−0.006
		Girls	—	—	—	—	−0.016	0.134	0.084	−0.016
Xerosis conjunctiva (Kodachrome)	Snohomish	Boys	—	—	—	—	—	0.016	—	—
		Girls	—	—	—	—	—	0.091	—	—
	Yakima	Boys	—	—	—	—	—	−0.072	0.088	—
		Girls	—	—	—	—	—	0.237	−0.001	—
Keratinization of upper arm Biomicroscopic)	Snohomish	Boys	—	—	—	—	—	—	—	−0.165
		Girls	—	—	—	—	—	—	—	0.041
	Yakima	Boys	—	—	—	—	—	—	0.079	−0.110
		Girls	—	—	—	—	—	—	0.363†	0.051

* Significant at 5% level.
† Significant at 1% level.

observations of the epithelium of the upper arm (xerosis, folliculosis, and hyperkeratosis) was 1.4 for the Yakima boys and 1.4 for the Yakima girls. These scores appear similar to the scores obtained for the biomicroscopic examination of the same tissue, for the same subjects. However, they are not directly comparable as a five-place scale was used in the biomicroscopic evaluation, and a four-place scale for the gross evaluation.

Physical examination as an aid in assessing nutritional status of adolescents has been used in studies conducted by Beach,[11] Odland and Ostle,[17] Babcock *et al.*,[28] and Trulson, Hegsted and Stare.[29]

CORRELATIONS

Simple correlation coefficients for nutrient intake, blood serum levels and subclinical symptoms are shown in Table IV.

Dietary Vitamin A: Vitamin A intake correlated with serum vitamin A was found to be nonsignificant for the Washington subjects. Likewise, no correlation was found by the workers in Idaho[8] and in the Northeast,[30] although a slightly positive relationship was found in Montana.[17] Highly significant positive correlations were found in Washington State for the girls from Snohomish County and the boys from Yakima County, for the vitamin A intake and serum carotene levels. Correlations were significant at the 5 per cent level for subjects in Idaho[8] and New York State[30] but not significant in Maine and Rhode Island.

Vitamin A intake from plant sources correlated with serum vitamin A was not significant for the subjects in Washington State. However, correlation of the intake from plant sources with serum carotene was significant at the 1 per cent level for all but the girls from

Yakima County, which was significant at the 5 per cent level.

Merrow *et al.*[31] found that vitamin A and carotene intake were reflected in their respective serum levels, and conversely the serum levels reflected the vitamin A and carotene intake. However, Caster and Mickelsen[32] concluded that the concentration of vitamin A in the blood is a characteristic of the individual rather than a reflection of the dietary intake of vitamin A. They recommend that the estimation of serum carotene be used as a measure of the vitamin A status. In this study, significant correlations were obtained between the dietary vitamin A and serum carotene but not between the dietary vitamin A and serum vitamin A.

Vitamin A intake positively correlated with serum vitamin C was significant at the 1 per cent level for Snohomish boys, at the 5 per cent level for Snohomish girls and Yakima boys, and not significant for Yakima girls. Workers in Maine and New York[30] found significant correlations, whereas workers in Rhode Island[30] found a highly significant correlation. Other correlations with vitamin A intake were not significant for the Washington subjects. Anderson and Milam[33] and Darby and Milam[34] found no correlation between the dietary intake of vitamin A and conjunctival changes in the eye.

The correlation of vitamin A intake from plant sources with serum vitamin C showed the same levels of significance as vitamin A intake with serum vitamin C. Other correlations with dietary carotene were not significant for these subjects.

The significant relationships between dietary vitamin A and serum ascorbic acid cannot be explained on the basis of similar food sources of vitamin A and ascorbic acid. Fifty-one per cent of dietary vitamin A was obtained from leafy, green and yellow vegetables. Tomatoes, citrus fruits and other fruits and vegetables contributed 14 per cent of the vitamin A in the diet. Other major contributing food groups: butter and fats, 11 per cent; meat products, 9 per cent and eggs, 6 per cent. Tomatoes and citrus fruits were the major food sources of ascorbic acid, contributing 38 per cent in the diet. The remainder of dietary ascorbic acid was almost equally supplied by milk products, leafy, green and yellow vegetables, other fruits and vegetables and potatoes.

Serum Vitamin A: Highly significant correlation coefficients were found for the subjects in Snohomish County and for the boys in Yakima County when serum vitamin A was correlated with serum carotene. The coefficient for the girls from Yakima County was not significant. Correlations were found to be highly significant by Babcock *et al.*[30] for Maine, New York and Rhode Island; and by Bring, Warnick, and Woods[12] for Idaho, Storvick, Hathaway and Nitchals[15] for Oregon, and Odland and Ostle[17] for Montana; Robinson *et al.*[35] in Michigan did not find a significant correlation.

Correlation coefficients for serum vitamin A and serum ascorbic acid were significant at the 1 per cent level for the girls in Snohomish County and Yakima County; and at the 5 per cent level for the boys of Snohomish County. No correlation was found for the boys in Yakima County, as was the case for subjects in Maine and Rhode Island[30] and Michigan.[35] Highly significant correlations were found by Storvick, Hathaway and Nitchals[15] in Oregon and Babcock *et al.*[30] in New York.

Correlation coefficients significant at the 5 per cent level were found for the Snohomish boys for serum vitamin A and xerosis of the bulbar conjunctiva, as judged from kodachrome slides; and for Yakima boys for serum vitamin A and the biomicroscopic observations of keratinization of the upper arm. All other correlations calculated with serum vitamin A were not significant for the Washington subjects. Anderson and Milam[33] and Darby and Milam[34] did not find a correlation with blood vitamin A and conjunctival changes.

Serum Carotene: Serum carotene correlated with serum ascorbic acid was highly significant and positive for all subjects, for both boys and girls. Highly significant correlations were also found by workers in Idaho,[12] Oregon,[15] Northeast Region,[30] and for the nonrheumatic girls in Utah.[16] The boys in Utah had a correlation significant at the 5 per cent level. No significant correlation was found for the

subjects in Michigan.[35] Babcock *et al.*[30] found a significant correlation for blood carotene with folliculosis of the upper arm.

In this study, positive significant correlations were obtained for serum vitamin A and serum carotene correlated with serum ascorbic acid. Animal studies[36] have shown that a metabolic relationship seems to exist between vitamin A and vitamin C. Ascorbic acid appears to influence the blood and tissue levels of vitamin A. Mayer and Krehl[37] found that when rats were maintained on a vitamin A-deficient diet, one of the symptoms of vitamin A deficiency was a decrease in the ascorbic acid level in the blood.

Biomicroscopic and Gross Observations: A highly significant correlation was found for the Yakima girls for the biomicroscopic observations of keratinization of the upper arm with the physical observations of keratinization of the upper arm. Serum vitamin C correlated with xerosis of the bulbar conjunctiva, as observed with the slit lamp, was found to be significant at the 5 per cent level for the Yakima boys.

Kruse[19] has stated; "It should be clear that there is not necessarily a high correlation between data derived by different methods on the same deficiency disease. They provide information on different aspects and states of the disorder." It has been theorized that the dietary intake contributes to the most recent state of nutrition, the blood findings reflect the immediate past history[19] and the condition of the body tissues more nearly reveal the long-term nutritional effects.

Pooled Simple Correlations: Pooled simple correlation coefficients were obtained from vitamin A in the diet correlated with: calories, carbohydrate, riboflavin, niacin, and iron in the diet; free and total riboflavin, and free and total cholesterol, sedimentation rate and hemoglobin in the blood; roentgenograms of the os calcis, phalanx-center and phalanx-end of the hand; cavities by exploration, number of missing teeth, caries index (number of surfaces involved in the decayed, missing or filled teeth) and number of decayed, missing and filled teeth (D.M.F.). None of these pooled correlation coefficients were significant for the boys or girls from either area.

Pooled correlations were also calculated for serum vitamin A and serum carotene with the same measurements as for dietary vitamin A.

Highly significant, positive pooled correlation coefficients were obtained between serum vitamin A and red blood cells, and serum vitamin A and hemoglobin in the blood. Bring *et al.*[12] did not find a significant correlation between serum vitamin A and hemoglobin. Correlations were significant at the 5 per cent level for serum vitamin A and the bone density determined with roentgenograms of the os calcis, and at the 1 per cent level for phalanx-center and phalanx-end of the hand. All these pooled correlations were positive. No evidence could be found in the literature to explain this relationship between serum vitamin A and bone density measurements. Significant, negative pooled correlations were obtained between the number of missing teeth, caries index and serum vitamin A.

Highly significant, positive, pooled correlations were obtained between serum carotene and the dietary intake of riboflavin, niacin and iron; and serum cholesterol, both free and total. The workers in Idaho[12] found positive, highly significant correlations for serum carotene and serum cholesterol; also, significant correlations between serum vitamin A and serum cholesterol. It is of interest that vitamin A, carotene and cholesterol, all fat-related compounds, show these relationships. Maddock, Wolbach, and Maddock[38] have said: "a fall in cholesterol and total lipids in the rat on vitamin A deficient diets, makes the implication that vitamin A is a factor in lipid metabolism a possible hypothesis."

Pooled correlations, significant at the 5 per cent level, were found between serum carotene and the number of missing teeth. Whereas, pooled correlations were significant at the 1 per cent level for serum carotene and the number of cavities found by exploration, caries index and number of D.M.F. teeth. All pooled correlations were negative. The relationship between dental caries and vitamin A nutrition has been summarized and reported by Bagnall[39] and Jeans.[40] It is interesting to

note that there is no definite evidence that dental caries are increased with a deficiency of vitamin A.

The dietary content of vitamin A indicates that the intake of these subjects, as a criterion of nutritional status was "excellent." However the other indices, blood level and gross and biomicroscopic observations, indicate that their vitamin A nutrition was "fair" to "good." It is of interest to note that one-half of the vitamin A in the dietary intake of the Washington subjects was provided as carotenoid precursors and the other half as preformed vitamin A. It is generally assumed that the ratio of carotenoid precursors to preformed vitamin A in the average diet is 2 to 1.[41]

Factors such as the ease of absorption and conversion of vitamin A precursors; vitamin A and carotene metabolic interrelationships with other nutrients; as well as the role played by the bodily stores of vitamin A, all influence the picture of vitamin A nutrition. In this particular study, the arbitrarily chosen criteria for the gross and biomicroscopic observations may have been set at too high a level to be practical. Had these criteria not been chosen with such high standards in mind, then better agreement might have been found between the subclinical signs of vitamin A malnutrition and the dietary intake.

SUMMARY

Vitamin A nutrition, for 248 15- and 16-year-old native-born and -reared Washington boys and girls, was assessed for two areas, Snohomish County and Yakima County. The indices used were: seven-day dietary record, serum vitamin A and serum carotene analyses, biomicroscopic observations of specific tissues, colored kodachrome photographs of bulbar conjunctiva, and physical observations.

No significant difference was found between the intakes of vitamin A of the 15- and 16-year-old subjects, either boys or girls. The intake of the boys was higher than that of the girls and this difference was highly significant. Area differences were not significant. No significant difference was found between serum vitamin A and serum carotene, for age, sex or area for these subjects. With the exception of the dietary content vitamin A, which could be classified as excellent, the other indices indicated the state of vitamin A nutrition as fair to good, for these boys and girls.

Simple correlations were calculated in an effort to establish the relationship between dietary intake, blood level and subclinical signs of a deficiency of vitamin A. Significant correlations were found for vitamin A intake with serum carotene and serum ascorbic acid; vitamin A intake from plant sources with serum carotene and serum ascorbic acid; serum vitamin A with serum carotene and serum ascorbic acid; serum carotene with serum ascorbic acid. Few correlations were found between the subclinical signs associated with vitamin A deficiency and fasting blood serum levels and dietary intake.

Pooled simple correlation coefficients were calculated for dietary vitamin A, serum vitamin A, serum ascorbic acid with diet nutrients: calories, carbohydrate, riboflavin, niacin and iron; with riboflavin, cholesterol, phosphatase in blood serum, and sedimentation rate, hemoglobin and red blood cells in whole blood; bone density measurements and observations of the teeth. Significant pooled correlations were obtained for serum vitamin A with: hemoglobin and red blood cells; density evaluation of the os calcis, phalanx-center and phalanx-end of the hand; number of missing teeth, and caries index. Significant pooled correlations were obtained for serum carotene with dietary riboflavin, niacin and iron; cholesterol, free and total in blood serum; cavities by exploration, number of missing teeth, caries index, and number of D.M.F. teeth.

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The Loss of Calcium, Phosphorus, Iron, and Nitrogen in Hair from the Scalp of Women

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WHEN BALANCE studies are used to assess the iron, calcium, phosphorus, and nitrogen requirements of human adults, the question arises as to how much of each element must be retained to cover the small losses. Among these losses are those from hair, including that on the scalp, on the skin and on axillary, pubic, and other areas. The present study was planned to determine the losses of iron, calcium, phosphorus, and nitrogen attributable to the loss of hair from the scalp. Determinations have been made of these four elements in hair by a few workers who have reported widely different values for the three minerals. As far as the authors have been able to ascertain, no study has been made of the weight of hair lost from the scalp.

PROCEDURE

Twelve women 18 through 22 years of age and one 54 years of age served as subjects. The young women collected hair combings daily over a period of four weeks with the exception of Sundays and the older subject for twelve six-day weeks. Each subject wore a hair net night and day and combed her hair in the laboratory while wearing a plastic cape and standing on a plastic sheet. All shampooing during the combing collection period was conducted at the laboratory and the water used in the process was strained through a cloth filter to catch the hair in it. Hair collected during shampooing was regarded as combings. Three hair trimmings were conducted at the laboratory for each subject who customarily had her hair cut. The plastic covers and sheets used during cutting were hung from a rod, the cuttings washed into a trough and the water filtered through a cloth. The hairs were picked off the plastic protectors and the cloth filters with tweezers. For the older woman the hair was collected from all shampoos, nine in number, from October, 1955 through May, 1956 and from two permanent waves. Her hair was cut only at the time the permanent waves were given and the collection represented all the cuttings for an entire year.

Most of the hair collection from each subject was washed twice in a 2 per cent soap solution made with hot distilled water, and thoroughly rinsed. The rest of the hair, enough for the iron determinations, was washed twice in a hot solution of a liquid synthetic detergent designated as "shampoo A." Two teaspoonfuls of shampoo A were used with each 100 ml of distilled water. The hair was allowed to soak in the shampoo solution overnight; it was then rinsed. The wash-waters were filtered through cloth. The tiny bits of hair which were collected on it were used in the determinations of the total weight of hair-loss but not for the determination of nutrients.

The rinsed hair was dried at 90 to 95° C until it reached a constant weight. The operation was time-consuming because the hair was so hygroscopic that drying was not effective on humid days. Three samples of hair of a weight appropriate for the particular nutrient or nutrients under study were placed in weighing bottles and the hair again heated to a constant weight. For the determination of nitrogen the hair was transferred to 100 ml Kjeldahl flasks; for the minerals it was transferred to platinum dishes and ashed at 500° C.

Nitrogen was determined by a semi-micro Kjeldahl method in which selenium was the catalyst; the ammonia was distilled with steam into a solution of boric acid. The ash for the

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determination of the mineral elements was dissolved in dilute HCl, then concentrated HCl was added. The solution was heated to dryness on a steam bath and the ash removed from the dishes with a solution of HCl. Iron was determined by the ortho-phenanthroline method using sulfurous acid for the reducing agent as recommended by Dutcher and Rothman.[1] Calcium was determined according to the micromethod for plants as given in the Official Methods of Analysis of the A. O. A. C. 1955.[2] Phosphorus was determined by the method of Simmons and Robertson.[3]

Determinations of the four nutrients were made on cuttings, except for the one subject who did not cut her hair. In her case all determinations were made on combings. A few determinations were made on combings collected from other subjects.

A few samples of the subject's hair and a few samples of hair obtained from a beauty parlor were used to study the effect of several commercial shampooing preparations and one soap.

In order to calculate the annual loss of hair records were kept of the date of each shampoo over a period of three months and of each of three consecutive trimmings. The assumption was made that the frequency of shampooing and trimming would be the same during the rest of the year as during the interval when records were kept.

RESULTS AND DISCUSSION

The total weight of hair lost in combing, shampooing, and trimming the hair from the heads of 12 young women varied from 41.36 to 105.96 g/year (Table I). The mean loss for the 12 subjects was 70.73 g. The two subjects with the largest losses had thick hair; the hair was also coarse as scored by five judges. The three subjects with the smallest losses had the finest hair. All losses of hair reported may be considered minimum because occasional hairs were no doubt lost through the hair nets.

The loss of 49.18 g/year by the subject who was 54 years of age, and who was included because more complete collections were available than for the younger women, fell within the range of the losses of the younger group. According to Myers and Hamilton[4] the hair on the scalp grows faster at ages 45 to 52 than at 21 to 30 years. According to Müller[5] hair does not grow to its full length after 35 years of age

TABLE I

Annual Loss of Hair from the Scalps of 13 Women and the Content of the Hair in Four Nutrients

Subject	Hair color	Content of 100 g of hair*				Annual loss†				
							Nutrients contained in hair			
		Ca *mg*	P *mg*	Fe *mg*	N *g*	Hair *g*	Ca *mg*	P *mg*	Fe *mg*	N *g*
P. F.	Dark brown	175	13.3	1.28	15.6	105.96	172	14.1	1.42	16.5
J. W.	Dark brown	104	17.5	1.67	15.8	103.32	108	18.1	1.72	16.3
J. H.	Blonde	205	11.4	1.95	15.2	87.12	179	9.9	1.70	13.2
S. W.	Light brown	198	12.9	1.52	15.9	86.59	172	11.2	1.31	13.8
S. T.	Red	224	11.2	2.43	14.5	78.27	143	9.1	1.76	11.4
H. C.	Medium brown	78	14.2	1.41	15.8	70.92	51	10.1	1.00	11.2
K. S.	Red	13	13.0	1.96	16.6	68.81	9	9.0	1.35	11.4
A. R.	Dark brown	45	13.5	1.35	16.4	57.57	26	7.8	0.78	9.4
N. V.	Red	62	17.2	1.39	15.9	52.72	33	9.1	0.73	8.4
S. K.	Blonde	58	14.2	1.53	16.2	48.24	28	6.8	0.74	7.8
A. S.	Medium brown	161	13.8	1.78	16.3	47.91	77	6.6	0.82	7.8
M. S.	Medium brown	200	11.5	3.28	14.7	41.36	83	4.8	1.36	6.1
Mean		127	13.6	1.80	15.7	70.73	90	9.7	1.22	11.1
S. D.		74.4	2.02	0.572	0.65	21.890	63.2	3.56	0.396	3.37
Z.	Medium brown and gray	71	13.3	1.24	15.7	49.18	26	6.5	0.72	7.7

* All values for calcium, phosphorus, and iron are for determinations made on cuttings except in the case of J. H. who did not cut her hair. Values for her hair were determined on combings.

† The annual loss was calculated from values for combings as well as cuttings in a few cases in which values for the nutrient content of combings was determined.

and Flesch,[6] in a review of hair growth, states that hair becomes larger in diameter with age.

In considering the total loss of hair the loss from parts of the body other than the head must be taken into consideration. Short hairs, the loss of which is too large to neglect, are found on all but a few areas. Myers and Hamilton[4] found that the period of regeneration and the life span of the hairs from the other areas are shorter than for hair on the scalp, but the rate of growth is slightly slower (except for hairs on the chins of men). The total amount of body hair varies widely with age and sex and from person to person.

The mean calcium content of the hair of the 12 subjects was 127 mg per 100 g. The content varied widely from subject to subject, from as little as 13 up to as much as 224 mg (Table I). Other workers, too, have found a wide range. Ikeuchi[7] noted a range of 65 to 157 mg and Goldblum, Derby and Lerner[8] from 70 to 490 mg. The low mean value of 20.8 mg found by Bagchi and Ganguly[9] may have been occasioned by the treatment with hot dilute HCl in the cleaning process. A reason for the wide range will be discussed later.

The phosphorus content ranged from 11.2 to 17.5 mg/100 g (Table I). This was a little higher than the range of 2.6 to 14.0 mg found by Goldblum, Derby, and Lerner,[8] but considerably below the mean of 80 mg found by Bagchi and Ganguly.[9]

The mean iron content of the hair from 12 subjects with blond, brown, and red hair was 1.80 mg/100 g; the range was from 1.28 to 3.28 mg (Table I). These values are far below that of 14.10 mg found by Bagchi and Ganguly[9] for black hair, and far higher than the values of 0.11 mg for blond and 0.68 for medium brown hair by Kikkawa, Ogita and Fujito.[10] The latter workers reported 2.9 mg for black hair. The values found in the present study are slightly higher than those of 0.08 to 1.1 mg of Goldblum, Derby, and Lerner[8] and slightly lower than the values of Dutcher and Rothman.[1] The latter workers reported 2.71 mg for black hair, 2.43 mg for blond hair, and a mean of 9.78 mg for three subjects with red hair. Red hair was found not to be exceptionally high in iron in the present study. A few samples were wet-ashed by the method used by Dutcher and Rothman,[1] but this did not alter the results.

The mean nitrogen content of the hair was 15.7 ± 0.65 g per 100 g. This is similar to values of 15.8, 16.3, 15.4, and 14.6 g found by Rutherford and Hawk;[11] Stary;[12] Clay, Cook, and Routh;[13] and Bagchi and Ganguly,[9] respectively.

In some subjects the color and texture of the cuttings differed from the combings. This suggested that a difference may exist in composition. Each hair has a growth period and then is retained for a while after it has severed connections with the hair follicle. Combings may contain a relatively high proportion of hairs which have ceased to grow, are older than the other hairs, and have been shampooed more times. The color difference was particularly striking in the case of subject S. T.; her cuttings were bright red and her combings a dull gray-brown. The calcium, phosphorus, and iron contents of her cutting were 224, 11.2, 2.43 mg/100 g, respectively, and of the combings 107, 12.5, and 1.90 mg. Since the difference in the calcium and iron content was large, samples from other subjects were studied. In the case of subject P. F. the cuttings contained 175 and the combings 142 mg of calcium per 100 g of hair. For two subjects the phosphorus content of cuttings and combings was similar. The difference between the amount of iron in the cuttings and combings of the other subjects was small; in one case the quantity of iron in the combings slightly exceeded that in the cuttings.

The mean annual loss of calcium, phosphorus, and iron in the combined combings and cuttings of the twelve young women was 90, 9.7, and 1.22 mg, respectively (Table I). This means that the average woman would need to retain from her food 0.35 mg of calcium, 0.04 mg of phosphorus, and 0.005 mg of iron a day to compensate for the loss. The mean annual loss of nitrogen was 11.1 g/year (Table I); thus each day 0.043 g would be lost and that amount would need to be retained from the food. Experience in this laboratory shows that young women retain approximately 0.6 g of nitrogen a day on intakes of about 60 g

of protein. About one-fourteenth of this would be required to cover the loss of nitrogen in the hair from the scalp. If the assumption is made that adult women are in nitrogen equilibrium when all the small losses are included, this figure is reasonable since additional losses include those in hair from other parts of the body as well as losses in nails, skin, perspiration, saliva, tears, nasal excretions, and menses.

The above values do not consider the possible loss of nutrients other than those present in the hair at the time it is cut or removed by combing. The removal of nutrients during shampooing is the most obvious possible other cause of loss.

A problem encountered during the study was that of how to remove calcium deposited on the surface of hair during shampooing in calcium-containing water, if such a deposition occurs, and yet not remove the calcium which is a constituent of the hair. If hair is soaked in dilute HCl almost all the calcium is removed from it; this was not done. In the early analyses made during the present study, the calcium content of hair washed in a 2 per cent soap solution was higher than that in hair washed with either commercial shampooing preparations A or B (Table II). In later studies in which the cuttings from subjects P. F. and J. W. were washed once in soap then five times in soap or shampoos A and E, about the same amount of calcium was removed (Table II). In the latter studies the treatment was rather severe: each of the six washings included stirring the hair in the washing solution, with two footed glass rods, lifting the hair to another dish, washing with fresh solution, and finally rinsing several times in distilled water.

TABLE II

The Effect of Shampooing on the Calcium, Phosphorus and Iron Content of Hair

Sample of hair*	Washing procedure	Ca	P	Fe
		Mg per 100 g of hair		
A	Once in soap	273	12.4	1.6
	Once in shampoo A	207	13.0	1.8
	Once in shampoo B	207	12.3	1.7
Z	Once in soap, then			
	No additional treatment	—	13.1	0.9
	Soaked overnight in shampoo A	—	11.4	1.1
	Washed 10 times in shampoo A	0	10.8	2.2
J. W.	Five times in shampoo A	36	14.5	1.4
	Five times in shampoo C	36	14.5	0.9
B	Soaked overnight in shampoo A	—	11.5	1.7
	Washed 5 times in shampoo A	—	11.5	1.9
C	Once in soap	329	—	—
	Once in shampoo A	305	—	—
D	Once in soap	104	12.1	0.8
	Once in shampoo D and sprayed with a curl set	38	12.2	0.9
P. F.	Once in soap, then			
	Five times in soap	69	10.0	1.06
	Five times in shampoo A	63	10.2	1.07
	Five times in shampoo E	58	10.7	0.96
J. W.	Once in soap, then			
	Five times in soap	34	12.9	1.00
	Five times in shampoo A	43	14.7	1.42
	Five times in shampoo E	38	14.1	0.92

* Samples A, B, C, D are cuttings obtained from a beauty parlor. The other samples are cuttings obtained from subjects.

The continued washing of hair with either soap or commercial shampooing preparations and distilled water reduces the calcium content. After washing once in soap the hair of subject P. F. contained 175 mg of calcium/100 g of hair (Table I) but after washing six times it contained 69 mg (Table II). With similar treatment the calcium content of subject J. W.'s hair fell from 104 to 34 mg. No experimental washings of cuttings were made in which hair was washed in tap water. Whether the calcium content of tap water would reduce the loss of calcium is not known.

If washing in solutions of shampooing preparations in tap water (the procedure used by the subjects when they washed their hair) removes calcium from hair, the subjects must have removed a considerable amount in that way since most of them washed their hair frequently. Of the 12 young women six shampooed their hair about once a week and the others more often. Subject K. S., whose hair had the lowest calcium content, washed her hair once a week with shampoo A and subject A. R. with the second lowest calcium content had been washing her hair every three or four days with shampoo D, then spraying it with a pin curl set. When samples of hair cuttings

obtained from a beauty parlor were washed with shampoo D in distilled water and rinsed with the set used by subject A. R., the calcium content was about a third of that found in samples washed once in soap (Table II).

The 12 subjects had been using nine different shampooing preparations; four subjects had been using a rinse or set in addition. No differences in the amount of calcium removed by commercial shampooing preparations were noted in the limited tests made in this study. Whether the pH of the washing solution had an effect on the amount of calcium removed is not known. One washing of cuttings in a soap solution made with distilled water left more calcium on or in the hair than the prepared shampoos. The pH of the soap solution was approximately 10 while that of shampoo A was 6 and that of the other preparations ranged from about 7 to 8.

The phosphorus content of the hair was reduced less than that of calcium by repeated washing procedures. After one washing in soap the phosphorus content of the hair of subject P. F. was 13.3 mg/100 g (Table I); after six washings it contained 10.0 mg (Table II). Similar treatment of cuttings from subject J. W. caused the phosphorus content to fall from 17.5 to 12.9 mg.

As far as iron is concerned, hair washed in shampoo A usually contained more iron than hair washed in soap. The differences were small except when hair was washed ten times in shampoo A which raised the iron content from 0.9 to 2.2 mg/100 g (Table II). The reason for this is not known. The shampoo contained only a trace of iron so the higher iron content of the hair could be accounted for only if practically all the iron in the solution migrated to the hair. Since washing removes fat and the samples were weighed after washing, some concentration of the iron content may have occurred. Why concentration might occur in the case of iron and not of phosphorus can only be explained by the removal of some phosphorus as well as fat during repeated washing.

Commercial shampooing preparations may affect the iron content of hair differently. The use of shampoo A gave the hair a slightly higher iron content than was obtained with shampoo B, but the difference was so small as to be within the error of the method (Table II). Shampoo A yielded hair containing considerably more iron than that found in hair washed with shampoo C but hair from only one subject (J. W.) was tested (Table II); the iron content of hair washed in shampoo A was also greater than that found when shampoo E was used, the increase being slight for one subject (P. F.) but considerable for another (J. W.) (Table II). Whether the use of soap or some other constituent or constituents in shampooing preparations accounts for the effect on the iron content of hair is not known. Shampoo A contained synthetic detergent only; B was part soap and part synthetic detergent; the composition of C and E is not known to the authors.

SUMMARY

Twelve women, 18 through 22 years of age, and one woman, 54 years of age, collected hair from the scalp. Combings for four six-day periods, shampoo losses during 28 days, and hair cuttings from three trimmings were collected by the young women. Collections over longer periods of time were made by the older woman. The mean annual loss of hair, after washing and drying at 90–95° C, was 70.73 g/person/year for the young women; the range was from 41.36 to 105.96 g. The older woman lost 49.18 g. The loss of hair reported here was minimum because a little undoubtedly escaped through the hair net worn during the combing-collection periods. The value does not include hair lost from other parts of the body.

The mean content per 100 g of hair for each of 12 young women was 127 ± 74.4 mg of calcium, 13.6 ± 2.02 mg of phosphorus, 1.80 ± 0.572 mg of iron, and 15.7 ± 0.65 g of nitrogen. The mean annual loss for each young woman was 90 mg of calcium, 9.7 mg of phosphorus, 1.22 mg of iron and 11.1 g of nitrogen. The annual losses of the older woman were within the range for the others.

The figures here reported for the annual loss of nutrients by way of the hair may be too small because some nutrients, especially cal-

cium, may be lost from hair during shampooing. The type of preparation used for shampooing may affect the loss of minerals: a certain soap removed less calcium than a certain commercial shampoo, when hair was washed once in each, and one commercial shampoo had the effect of either adding iron to hair or causing the retention of more iron than soap and certain other commercial shampoos.

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The Metabolism of Serum Albumin in Man During Brief Starvation

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PROTEINS which are eaten, digested, and absorbed face only two alternatives: incorporation into tissue protein or catabolism to nitrogen, carbon, and sulfur residues. However, when plasma proteins are administered by vein, a third possibility exists: the proteins may circulate unaltered in the blood, lymph, and interstitial fluid for significant periods of time.[1,2] It is for this reason that a short-term positive nitrogen balance following parenteral administration of plasma proteins does not prove their incorporation into tissue proteins. Studies from this laboratory[3] indicated that the magnitude of this metabolic "dead space" following albumin administration was considerable. The present investigation was designed to secure information as to whether the catabolism of albumin increased under conditions promoting the catabolism of tissue protein in general. Starvation was employed, since it is a potent stimulus to the breakdown of protein so that glucose may be made available.

Experiment: The experiment was planned to compare the metabolism of amino acids and of albumin under similar circumstances. The volunteer subject was a healthy 23-year-old medical student, 6 feet, 3 inches tall and weighing 87 kg. Except for the test protein materials the subject took no nourishment of any kind during the study; drinking water was not limited. A multivitamin capsule and 4 g of sodium chloride were given daily. Activity was restricted, in part by weakness.

The first portion of the experiment consisted of three periods:

(1) Fast, four days.

(2) Daily administration of 25 g of amino acid nitrogen* given intravenously in two divided doses—two days.

(3) Fast—three days.

A month was then allowed to elapse for the subject to regain his normal nutritional state. During this month, other experiments in albumin metabolism[3] were carried out in the course of which subjects developed congestive failure with a week's time from receiving 75 g intravenous albumin daily. It was clearly unsafe to administer the isonitrogenous dose of 150 g a day (25 g of albumin nitrogen) projected for the second portion of the experiment; for this reason, the two portions of the study are comparable but not identical. The second part of the experiment therefore consisted of the following three periods:

(1) Fast—four days.

(2) Daily administration of 12 g of albumin nitrogen† given intravenously in two divided doses—four days.

(3) Fast—three days.

Studies: The subject was weighed daily. 24-hour urine collections were made. These were analyzed for total nitrogen, urea, potassium, and inorganic sulfate as in a previous study.[3] A qualitative test was done for acetonuria. Determination of the respiratory quotient was made on several occasions, using 20-minute gas collection periods.

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* A lyophilized supplemented acid hydrolysate of casein containing alpha amino acid nitrogen as 75 per cent of the total nitrogen. A more extensive description of this material is provided by Hoffman *et al.*[4] Our supply was provided by the manufacturer, The Interchemical Corporation, of Union, New Jersey.

† 75 g of human serum albumin with 3 g DL-acetyltryptophan, supplied by the American National Red Cross.

RESULTS

The experiment progressed without untoward events except for the sixth day of the albumin study, when the subject developed a headache and temperature of 101° F. Since the effects of fever and starvation upon protein breakdown should be similar, this unforeseen complication should not interfere with the interpretation of the study. Acetone was consistently found in the urine after the first day, indicating that the subject was under an adequate stress to encourage protein breakdown and the formation of endogenous glucose. Respiratory quotient determinations varied from 0.69 to 0.72; there was no increase on the second day of amino acid administration.

Figure 1 and Table I permit a comparison of the amino acid and the albumin experiments. After amino acid administration urinary nitrogen doubled. In view of the output of urea, only a small fraction of this could have represented aminoaciduria. After albumin administration, in contrast, urinary nitrogen did not increase. The retention of sulfur during the albumin experiment in contrast to the amino acid study confirms the nitrogen balance trends. It is apparent that even under the stress of starvation albumin is not readily catabolized.

It is of interest to infer the proportion of administered albumin that was catabolized during the study. Because albumin contains 2 per cent sulfur[5] while most body and dietary protein have only 1 per cent, the extra associated sulfur constitutes a natural tag, the ratio of nitrogen to sulfur in the urine indicating the proportion of these elements derived from albumin. Urea and inorganic sulfate have a comparable status in the urine, each reflecting exogenous metabolism to a greater extent than the continuing metabolism in protein turnover,[6] and it is the ratio of these elements that is given. The ratios suggest that although nitrogen excretion following albumin failed to increase, a large fraction of the urinary nitrogen present both during and after albumin administration did arise from albumin rather than from other body tissues.

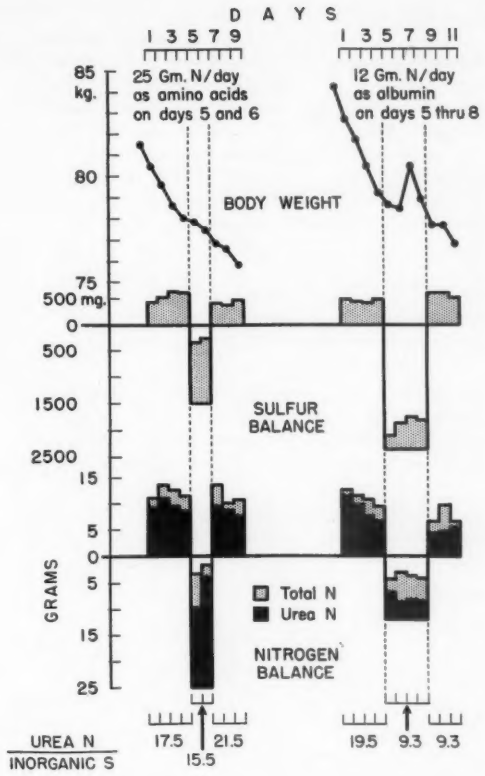

Fig. 1. In the portions of the graph showing sulfur and nitrogen balance, the method of Albright is employed; intake is indicated by a line below the baseline and output is charted upward from the intake line.

DISCUSSION

In normal subjects with normal amounts of circulating albumin, studies with I^{131}-labeled albumin have indicated a half-life of 10 days for this protein.[7] Similar studies with N^{15}-labeled albumin indicated a half-life of not more than 20 days.[8] The question for investigation has been whether excessive amounts of albumin will undergo more rapid catabolism under the influence of stress.

After phlorhizinizing dogs, Howland and Hawkins[9] administered plasma orally and intravenously. Although oral plasma was followed by increases in urinary nitrogen and glucose that made evident the availability of ingested plasma for gluconeogenesis, parenteral infusion was followed by no increases. It was concluded that even under marked carbohydrate deprivation the plasma proteins were

TABLE I

Comparison of Amino Acid and Albumin Experiments

Day	Intake		Urinary excretion				Ratio, urea nitrogen/ SO_4 sulfur, for period
	Nitrogen *g*	Sulfur *mg*	Total nitrogen *g*	Urea nitrogen *g*	Potassium *meq*	SO_4 sulfur *mg*	
Amino acid study							
1	—	—	11.4	9.1	65	430	
2	—	—	13.2	11.0	31	520	
3	—	—	11.6	9.6	39	590	
4	—	—	11.2	8.4	45	590	17.5
5	24.1	1,440	21.0	18.3	28	1,160	
6	25.4	1,520	23.7	21.2	15	1,230	15.5
7	—	—	13.3	9.7	6	400	
8	—	—	10.0	8.0	7	360	
9	—	—	10.5	7.4	15	440	21.5
Albumin study							
1	—	—	12.4	11.5	75	460	
2	—	—	11.2	9.4	32	460	
3	—	—	10.8	7.5	29	400	
4	—	—	9.4	6.6	35	310	19.5
5	12.2	1,500	7.7	5.5	24	300	
6	12.2	1,500	8.6	3.6	48	450	
7	12.2	1,500	7.7	3.9	25	590	
8	12.2	1,500	7.3	3.8	13	540	9.3
9	—	—	7.8	4.4	8	550	
10	—	—	9.2	4.7	5	560	
11	—	—	7.9	5.5	6	500	9.3

not available for degradation. The patients whom Fletcher *et al.*[10] infused with human serum albumin under the stress of a recent major operation also failed to increase their urinary nitrogen, unlike similar patients given protein hydrolysates.[11]

The present study indicates that even in starvation albumin continues to be slowly metabolized. It seems reasonable to conclude that either the system for the catabolism of excess albumin is limited, or it is insensitive to the methods of provoking it that have been attempted. It is interesting to speculate upon the proper interpretation of the precipitous drop in the urea nitrogen/inorganic sulfate sulfur ratio following albumin administration. Although it probably represents albumin catabolism, it might also represent rejection of sulfur-containing amino acids by the tissues incident to transformation of albumin into the proper composition of cellular proteins. Tagging of the nitrogen would be necessary to obtain a conclusive answer.

SUMMARY AND CONCLUSIONS

Evidence of protein and amino acid catabolism was sought for in a starving healthy subject who received intravenously human serum albumin in one experiment and an amino acid mixture in another. Following administration of the amino acid mixture the urine contained almost equivalent amounts of nitrogen and sulfur. In contrast, although some catabolism of albumin occurred, the majority of the albumin was retained by the subject. This finding indicates that even under the stimulus to protein catabolism posed by starvation, the rate of albumin breakdown is relatively slow.

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Ascorbic Acid Requirements of Adults: 30 mg or 75 mg?

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IN WESTERN Europe and North America it is possible to appraise an ordinary mixed diet if we know only the daily allowances of calories, protein, vitamins A and D, thiamine, riboflavin, niacin, ascorbic acid, as well as calcium, phosphorus, and iron, and it is for these nutrients that we need standards of requirement.

We have three main types of standards for daily requirements of nutrients:

(1). *Minimum requirement* indicating the quantity of a nutrient the daily allowance of which is sufficient to prevent the appearance of symptoms of disease.

(2). *Optimum requirement* indicating the quantity of a nutrient the daily allowance of which ensures for the individual the best possible state of health, functional efficiency, and resistance, e.g., to infections.

(3). *U. S. Recommended Dietary Allowances* do not quite correspond to the optimum requirements mentioned under point 2, but are stated to have been calculated so amply that they will under all circumstances cover the requirements of all healthy individuals, as well as cases where these are especially great, for instance, during temporary stress.

We know *the minimum requirements* best. Our first recognition of the importance of the qualitative and quantitative composition of the food is, indeed, due to the practical experience which shows that the lack of certain types of nutrient in the diet will cause diseases, and that allowance of the food and nutrient in question will make the disease disappear.

In Western Europe and North America, however, the minimum requirements have mainly theoretic interest. What we wish to know and compare with our dietary surveys are optimum requirements.

The reason why I have chosen to discuss optimum requirements of adults for ascorbic acid is that considerable disagreement exists about this very requirement. The fact is that whereas there is agreement on recommending a daily allowance of 30 mg ascorbic acid for infants, *The British Medical Research Council*[1] and a number of scientists in Northern Europe are of the opinion that about the same quantity is also the optimum requirement of healthy adults (apart from pregnant and nursing women), while *National Research Council* (of the U. S. A.)[2] recommends a daily allowance of 75 mg ascorbic acid for healthy adults.

The National Research Council has based and maintained the recommended dietary allowance of ascorbic acid at 75 mg for adults with reference to the comprehensive literature, which shows:

(1) There is evidence that minimum requirement may not be satisfactory for the preservation of optimum health through long periods of time, or when the body is subjected to common forms of stress.

(2) Reference is made to analyses of the contents of ascorbic acid in mother's milk, where the diet of the mother is "good."

(3) Tests with humans and animals of the quantity of ascorbic acid that will produce satisfactory healing of wounds, enzyme action, and cell proliferation.

(4) The variation of concentrations of ascorbic acid in various organs at different allowances of ascorbic acid.

(5) The recommended dietary allowances are about three to seven times greater than the quantities that will protect children and adults

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against macroscopic manifestations of scurvy, *i.e.*, minimum requirement.

(6) It is pointed out that the recommended dietary allowance of ascorbic acid is not "saturation" value since greater intake will produce higher concentrations in the tissues.

Another view is presented by the *British Medical Research Council*'s investigations in 1944–46, which form the basis of the insistence of the British on the requirement of the *League of Nations*,[3] namely, an allowance of 30 mg vitamin C a day for adults. The tests were conducted on 18 young men and one young woman with application of a thorough and varied research technic, i.e., analyses of the ascorbic acid content of plasma, leucocytes, and urine, investigation of healing of wounds, and observations of physical and mental efficiency.

The results may be summarized as follows:

(1) In order to attain a uniform nutritional state for all 19 subjects, they were given a test diet without supplements for six weeks. The test diet was ample in respect to calories and content of other nutrients, but contained only 1 mg ascorbic acid. After the six-week test period the subjects were divided into the following groups: (a) Ten persons continued on the test diet without supplements. After 26 weeks six of these ten developed hemorrhages of the skin and mucosa; moreover, after 35 weeks nine of the ten had pronounced changes in the gums, and artificially inflicted wounds showed slower and poorer healing.

(2) After six weeks on the basic diet, seven subjects received a daily allowance of 10 mg ascorbic acid. Examination showed no abnormal changes in any of them within a period of 160 days. The latter group was then divided into two subgroups, (a) Three of the seven subjects continued with a supplement of 10 mg ascorbic acid for another 92 and 264 days. All the methods of examination used showed continued normal conditions. (b) Three of the seven subjects received no supplement any more after the 160 days. After 71 days (10 weeks) light skin changes (keratoses) appeared, which disappeared when a daily allowance of 10 mg was again given.

(3) After the six-week period, three persons received a daily supplement of 70 mg ascorbic acid. Examination disclosed nothing abnormal in these subjects.

From these investigations the British Medical Research Council draws the following conclusions:

(1) 10 mg ascorbic acid a day for healthy adult persons on a diet containing no ascorbic acid but being otherwise optimal will cure scurvy and prevent the appearance of symptoms of scurvy over a period of observation of 12 months.

(2) The minimum requirement for vitamin C in adults must consequently be somewhat below 10 mg a day, and some test results seem to indicate that it is about 5 mg a day.

(3) With a daily allowance of 30 mg ascorbic acid it must consequently be assumed that we are on the safe side, and the optimum requirement is covered.

(4) Thus the daily allowance of 75 mg ascorbic acid recommended by the Americans for adults (not including pregnant and nursing women) should be too high.

The foregoing is a summary account of the opinions on the requirements for ascorbic acid.

The most important of the investigations that may form the basis for a determination of the most probable optimum requirements is the investigation of the requirements of ascorbic acid of guinea pigs and humans (Table I).

STUDIES ON GUINEA PIGS

Numerous tests with guinea pigs have shown that *macroscopic* symptoms of scurvy do not occur if the animal is given a daily allowance of 0.5 mg ascorbic acid (Table I). *Microscopic* scorbutic changes to the teeth of the guinea pigs can only be prevented by an allowance three to four times as great, i.e., 1.5 to 2.0 mg a day. If the guinea pigs are to show the greatest possible resistance to outer influences however (a question that has been examined by determination of healing speed of an artificially inflicted wound or bone fracture,[4] or by the degree of resistance to injection of diphtheritic toxin),[5] the guinea pigs must have a daily allowance of 5 mg ascorbic acid,

TABLE I

Standards and Requirements of Ascorbic Acid for Humans and Guinea Pigs

	Guinea pigs *mg*	Infants *mg*	Adults with requirement of 2,400 calories (except pregnant and nursing women) *mg*
Standards			
League of Nations 1938[3]	—	30	30
Brit. Med. Res. Council 1948[1]	—	30	30
U. S. Recommended Dietary Allowance, 1953[2]	—	30	75
Minimum requirement			
On the basis of macroscopic examination for scurvy	0.5	3–6	Below 10
On the basis of microscopic examination for scurvy	1.5–2.0	?	?
Optimum requirement			
Determined by resistance to infection and intoxication	5	?	?
Determined by healing of wounds and bone fractures	5	—	10

i.e., *ten times the dose that prevents macroscopic manifestations of scurvy.*

The fact that no symptoms of scurvy have been ascertained by means of the methods used, by which the minimum requirements have been fixed, does not prove that the guinea pig has been healthy. Perhaps it only indicates that our general clinical methods of examination are too coarse to disclose slight morbid manifestations of vitamin C deficiency. This assumption is supported in part both by tests with animals, and partly—as we shall see later—by observations on humans.

By tests with guinea pigs we find, as already mentioned, that 0.5 mg ascorbic acid a day will protect against the appearance of macroscopic indications of scurvy (changes in the cartilage of the ribs, visible hemorrhages, loose teeth, etc.) whereas as much as 0.5 to 2.0 mg a day is necessary in order fully to prevent the presence also of microscopic deficiency symptoms in the teeth of the animals. On the basis of a gross examination we should say that the minimum requirement of the guinea pig for ascorbic acid is 0.5 mg ascorbic acid a day, but after the microscopic examination that it is at least three to four times as great, viz., 0.5 to 2.0 mg. Thus even in the fixing of minimum requirement in guinea pigs a considerable uncertainty manifests itself.

STUDIES ON MAN

Investigations of the ascorbic acid requirement of *humans* are numerous, and only certain main points are mentioned here.

In *infants* nourished on diluted cow's milk, mild symptoms of scurvy are not rare.[6] Raw cow's milk contains from 0.1 to 2.5 mg ascorbic acid/100 ml, a little less than half when pasteurized. If the consumption of liquid by the infant during the first three months is estimated at 500 to 1,000 ml a day, and if the usual dilutions of the milk are used, a bottle-fed infant will get an average daily allowance of 3 to 6 mg ascorbic acid, and 3 to 6 mg thus will not in all cases be sufficient against the manifestations of scurvy. Consequently *the minimum requirements for infants* would seem to be greater than 3 to 6 mg ascorbic acid.

In this connection I should like, however, to call attention to the imperfection of our clinical examination technic in infants as pointed out by Follis *et al.*[7] Scurvy was diagnosed by microscopic examination of the bones of 69 dead children, although this ailment had been diagnosed clinically in only six of the 69 children while they were patients in the pediatric ward. It is therefore very possible that the minimum requirement for ascorbic acid if determined by a more elaborate technic is higher than the 3 to 6 mg stated for infants.

Mother's milk contains about four times as much vitamin C as cow's milk, which moreover is diluted in milk mixtures, in fact, fluctuating according to the diet of the mother from 4 to 8 mg/100 ml breast milk. Breast-fed infants, who never show indications of scurvy, thus get a daily allowance of at least 20 mg, often 40 to 50 mg ascorbic acid. Of course we do not know *the optimum requirement of infants* just because we know the quantities of ascorbic acid that breast-fed infants

receive in mother's milk, but it must be supposed nevertheless that mother's milk from healthy women living on a satisfactory diet is appropriately composed with regard to the nutrients that are essential for the infant, including ascorbic acid. This is supported by Braestrup's[6] analyses of the plasma ascorbic acid content in the blood and milk of nursing women, and the plasma and urine ascorbic acid in their children. The ascorbic acid content of the blood plasma of the mothers and of the mother's milk showed the usual seasonal variations whereas a corresponding seasonal variation was not demonstrated in the plasma of breast-fed infants, a fact which Braestrup construes as an indication that breast-fed infants are saturated or nearly saturated with ascorbic acid even in the poor season. This is in accord with the fact that a considerable number of the normal infants excreted ascorbic acid in the urine.

It is of interest, that in 50 of 53 healthy breast-fed infants in the first six months of life Braestrup[6] found plasma ascorbic acid in quantities between 0.5 and 1.5 mg per 100 ml, most often about 1.0 mg per 100 ml. In healthy infants who had been fed solely on diluted cow's milk, the plasma ascorbic acid contents averaged about 0.25 mg per 100 ml.

It is on the question of *the optimum requirement of healthy adults*, that disagreement prevails. The investigations are numerous. I shall only remind the reader of the observations of the British physician, Lind, in 1753 to the effect that scurvy might be prevented by a daily allowance of 25 to 30 ml lemon juice, which, according to our present knowledge, contains about 14 mg ascorbic acid.

Concerning the optimum requirement I wish to call attention to the results of the investigations of plasma ascorbic acid in test subjects, and to compare them with the previously mentioned investigations of plasma ascorbic acid of infants. These are indicated in Table II.

Whereas plasma ascorbic acid in persons on a freely chosen diet shows considerable variation, including pronounced seasonal variation, the plasma ascorbic acid is relatively constant in test persons living on a diet with a constant content of vitamin C provided that a fasting blood specimen is examined. It is seen that 50 mg ascorbic acid a day for adult test subjects will produce plasma ascorbic acid levels which are only a little above that of the bottle-fed infants (about 0.25 mg per 100 ml). Test persons who received 70 mg ascorbic acid a day had plasma ascorbic acid of 0.55 mg per 100 ml or barely as much as minimum of breast-fed infants.

The following reasons seem to indicate that the daily allowances recommended by Americans of 75 mg ascorbic acid for healthy adults with a daily requirement of 2,400 calories is closer to the optimum requirement than the 30 mg indicated by the League of Nations and by the Medical Research Council in England.

(1) The optimum requirement for ascorbic acid for guinea pigs is about 10 times the minimum requirement as determined by macroscopic examination.

TABLE II

Comparison of Plasma Ascorbic Acid in Adults and Infants

Daily allowance of vitamin C *mg*	Infants (*Braestrup*)[6]		Adults (*Brit. Med. Res. Council*)[1]		
	Diet	Plasma ascorbic acid *mg/100 ml*	Number of persons	Test period (days)	Plasma ascorbic acid *mg/100 ml*
1	—	—	10	205–269	<0.03
6	Bottle-fed infants (about 3–6 mg ascorbic acid a day)	About 0.25	3	125	<0.05
10	—	—	6	101–157	<0.10
20	—	—	5	47–92	<0.10
50	Breast-fed infants (30–50 mg ascorbic acid a day)	About 1.0 (0.5–1.5)	2	61–66	0.31
				—	
70	—	—	3	300–336	0.55

(2) Infants' allowance of ascorbic acid in mother's milk is about 10 times the minimum requirement, and the plasma ascorbic acid content is "locked" above 0.50 to 1.5 mg per 100 ml, as a rule about 1.0 mg per 100 ml.

(3) The minimum requirement of adults is not known precisely. It is between 5 to 10 mg ascorbic acid a day. The plasma ascorbic acid content in test persons receiving a daily allowance of 10 mg and 20 mg ascorbic acid was inconsiderable, below 0.10 mg per 100 ml and 50 mg ascorbic acid a day for adults gave only 0.31 mg per 100 ml. Only with 70 mg ascorbic acid a day were values attained that approach those known for breast-fed infants, i.e., more than 0.50 to 0.60 mg per 100 ml. Even at these plasma values the organism is far from being "saturated," i.e., from excreting ascorbic acid in the urine.

The methods of examination that can be applied to humans apparently are so coarse that all that can be established on the existing basis is that adults perhaps have a minimum requirement of 5 to 10 mg ascorbic acid a day when otherwise subsisting on a diet that is adequate in all other respects, and when leading a life without special exertions or harmful influences. If the minimum requirement has been determined with certainty to be about 5 to 10 mg of ascorbic acid a day, the optimum requirement of adults, on the basis of experience gained by investigations with guinea pigs and infants, should for the present most safely be estimated by multiplying by ten. Hence, the optimum requirement of adults must be estimated at 50 to 100 mg ascorbic acid a day. This leads to the American figure of 75 mg, which brings the plasma ascorbic acid up near that which we find in breast-fed infants without causing "saturation."

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Nutritional Adaptation to Low Dietary Intakes of Calories, Proteins, Vitamins, and Minerals in the Tropics

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THE IMPORTANCE of proteins, vitamins, and minerals in human nutrition has been the subject matter of extensive work.[1] Various authorities on food and nutrition have recommended standards of dietary intakes of these substances for different age groups and under varying physiologic conditions.[2] In underdeveloped countries like India it seemed somewhat doubtful whether such high intakes could be attained for economic reasons. A study of the nutritional adequacy of some selected sections of the population was, therefore, considered desirable.

MATERIAL AND METHODS

The present study comprises 240 subjects divided into two groups. Group I, representing a better dietary standard, included 80 medical students, 40 males and 40 females, (age 18 to 25 years) whereas Group II, representing a very poor dietary standard, consisted of 160 beggars including 40 men (age 28 to 46 years), 40 women (age 28 to 40 years), 40 children (age 2 to 15 years), 20 pregnant and 20 lactating women (multipara, age 19 to 38 years).

The procedure consisted of: (a) assessment of the diet of each individual; (b) clinical evaluation of physical and nutritional status; (c) radiologic examination of certain bones.

Assessment of Diets

After considering the special circumstances, detailed herein, complicating the proposed study, it was decided to obtain the average daily consumption of foodstuff from the total consumption calculated for several years, the basis for which was the current dietary intakes and dietary habits as well as past dietary history.

The diet of medical students was available for free inspection and weighing. Also, a fairly accurate record of the past dietary history could be obtained easily. The total consumption of foodstuffs of messes of groups of students was determined for two weeks at 15-week intervals. From this the average daily consumption was deduced. Additions were made for extra food taken outside the mess. From these data the monthly consumption was calculated.

The assessment of the diet of beggars posed many difficulties. Inspecting and weighing the diet was frequently impossible because of the peculiar mode of life. The street beggars in India are found in large cities. With their children they beg for food or money during the day. They are homeless and sleep on the roadside or foot paths or in abandoned buildings. They are usually underclothed. They have no fixed meals; instead they eat the food that they beg as often as they get it. Under such circumstances it was almost impossible to inspect and weigh the diet regularly.

The beggers were promised a full meal once a week for four weeks on fixed days. They were instructed to produce samples of the consumed articles for inspection. They were also questioned about the amount and composition of diet consumed during the preceding seven days. The maximum eating capacity was also recorded. Inquiries were made into

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the daily, weekly, monthly, and yearly incomes in each case to gauge purchasing capacity. The fact that the beggars ate piecemeal was always kept in mind in order to avoid minimizing the intakes. From these data and a consideration of all the important points obtained in cross-examination the weekly and fortnightly consumption was deduced.

The average yearly consumption of individuals in both groups was calculated from the data of monthly consumption, again after considering the monthly and seasonal variations in the diet. Inquiries were made regarding major changes during the preceding years. An attempt was made to judge the memory of each subject. Interrogation regarding the dietary history was adjusted accordingly. As the diet of the beggars did not vary much owing to extreme poverty, they could describe the amount and composition of their diets for the preceding two to five years. After applying the necessary corrections, the total consumption for a certain period of years was calculated and from this the average daily consumption was deduced. Rich diets at feasts or during convalescence from illness were not taken into consideration.

Allowance was made for waste in the diet of medical students to the extent that it was thought reasonable on the basis of the individual statements. Diets used during the period of febrile illness(es) were not taken into account nor were foods eaten only occasionally in small quantities. In the case of children the inquiries were directed to their relations or guardians. For pregnant and lactating women, the average daily consumption of foodstuff was calculated from the total for the duration of current pregnancy and lactation only.

The chemical values of various foodstuffs were calculated from the tables in the Health Bulletin No. 23, 4th edition, 1951, published by the Government of India. The following procedure was adopted in computing the chemical values.

The values for different cereals were separately calculated, whereas the values for various pulses, vegetables and fruits have been "pooled" for each individual or for a group of individuals consuming similar articles. If several articles of the same kind were used irregularly the chemical values of all such articles were "pooled." Hydrogenated fats and butters are included under the common heading of "ghee" (clarified butter). The vitamin content of the hydrogenated fats has been taken into account. Milk products have been calculated in terms of "khoya." The chemical values for spices have been pooled to obtain an average value.

The pooling method was also adopted elsewhere, when it was not possible to assess a correct amount or proportion.

Clinical and Radiologic Examinations

This included general data such as age, caste, dietary habits, occupation, a personal and family history regarding incidence of deficiency states and records of height and weight. There were also thorough systemic examinations for the presence of active or residual signs of rickets or osteomalacia or other deficiency states on the basis of the guidelines prescribed by Jolliffe[3] and Youmans.[4] Besides the established symptoms and signs of frank deficiency states the following key signs listed against various deficiencies were sought specifically:

(1) Calorie —height and weight tables
(2) Protein —dependent edema
(3) Vitamin A —follicular keratosis, xerosis, xerophthalmia
(4) Vitamin D —craniotabes
—rickety rossary
—deformities of legs, pelvis, and chest
(5) Thiamine —absent tendon Achilles reflexes, calf-muscle tenderness
(6) Niacin —hypertrophic or atrophic papillary lesions of tongue
(7) Riboflavin —angular stomatitis; conjunctival hyperemia; magenta tongue
(8) Ascorbic acid—hyperemic gums, perifolliculosis

The bones of wrist and pelvis were x-rayed to detect radiologic manifestations of deficiency states.

RESULTS

Clinical and Radiologic Examinations

Group I: The subjects were young medical students ranging from 18 to 25 years of age. Systemic examination did not reveal any evidence of clinical or subclinical manifestations of deficiency states. Carious teeth was detected in only one subject.

Group II: Of the 160 beggars, deformed chest in one man, kyphosis in one woman, deformity of lower extremity in one pregnant woman and carious teeth in two men, were noted. One male child had active rickets. Frank beriberi was noted in one man and there was suggestive evidence of multiple vitamin B deficiency in two men and one child. Follicular keratosis and night blindness were detected in two subjects. No evidence of ascorbic acid deficiency was available. No clinical or subclinical signs of deficiency states were detected in the remaining subjects.

Radiologic examination also showed active signs of rickets in the child in whom rickets was diagnosed clinically. There was no evidence of active rickets or osteomalacia in the skiagrams of other individuals of either group. The trabeculae of the bones were well developed.

A comparison of average heights and weights of the adults of the two groups and the mean values of heights and weights of American boys and girls 17 years of age, is given in Figure 1. In comparison to the American standards the average heights and weights of Indian adults of both groups are quite low. There is no significant difference between the average heights of adults of the two groups. The average weight of adult males of Group II is 24.5 per cent less than the average weight of male adults of Group I. The age-height and age-weight relationships of boys and girls of Group II were significantly lower in comparison to the American and English standards.

Analysis of Diet

Table I summarizes the data regarding the average consumption of various foodstuffs by the individuals of the two groups. It will be noticed that the bulk of the diet consists of cereals and pulses. The quantity of vegetables per consumption unit for Group II is less than 1 oz per day. Even in the diet of the medical students the consumption of fruits is only 1 oz per person per day. It is also evident that the diet of Group II is highly deficient in protective foods and foods of higher mineral and vitamin content such as milk, milk products, fruits, vegetables, meat, and eggs.

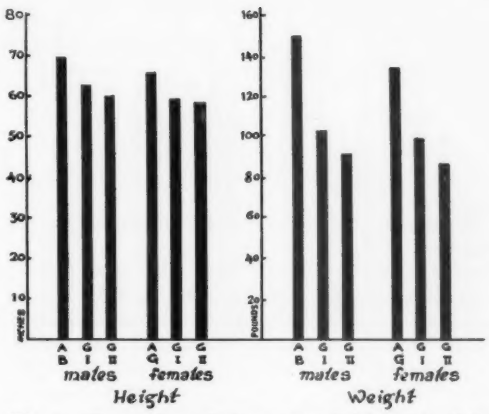

Fig. 1. Comparison of the average height and weight of adults of the two groups and American boys and girls of 17 years. $\frac{A}{B}$ = American Boy. $\frac{A}{G}$ = American Girls. GI-II = Group I–II.

The total caloric value of the diet and the average daily intake of proteins, vitamins and minerals as determined from the daily consumption of foodstuffs are presented in Table II. The total caloric value of the diets is quite low. The average daily intake of animal proteins even in case of Group I is 13.6 g. The diet of Group II contains negligible amount of animal proteins. Not even in the diet of a single individual out of the whole series was the daily intake of vitamin A satisfactory. The intake of thiamine and riboflavin was also unsatisfactory, especially in view of the high-carbohydrate diet. The diet of Group II is also meager in ascorbic acid content, while the diet of both the groups is lacking in vitamin D. It is remarkable that the average daily intake of vitamin D, even in the diet of growing children is less than one I.U.

The average intake values of iron are low. The daily calcium intake of adults in Groups I and II fluctuated between 763 mg to 1,067 mg and 208 mg to 375 mg, with an average of

TABLE I

Average consumption of Foodstuffs per Consumption Unit (in ounces)

Subjects	Total cereals	Wheat	Barley	Maize	Rice	Pulses	Vegetables	"Ghee" (pure butter fat)	Oil	Fruits	Milk	Milk products	Meat	Spices
Group I														
Adults	17.3	13.8	—	—	3.0	2.1	7.0	0.3	0.13	0.96	4.9	1.0	—	0.28
Group II														
Adults	16.2	6.4	4.8	2.9	1.3	1.9	0.5	0.10	0.11	—	—	—	—	0.03
Pregnant women	13.6	5.8	6.2	1.2	0.5	1.4	1.2	0.14	0.18	—	0.3	0.12	0.6	0.13
Lactating women	20.1	10.2	6.9	2.7	0.18	1.8	1.3	0.10	0.22	0.3	0.8	—	0.7	0.08
Children	9.1	4.1	3.1	0.8	1.9	0.7	0.8	0.68	0.13	—	—	0.06	—	0.02

928 mg, and 276 mg respectively. The calcium intake of medical students approached standard requirements. No extra calcium was available to children and adolescents for growth or to women undergoing the stress and strain of pregnancy and lactation. The average daily intake of calcium of children and adolescents, pregnant and lactating women, of Group II was respectively 173 mg, 319 mg, and 436 ranging between a minimum and a maximum of 141 mg to 234 mg, 280 mg to 378 mg and 372 to 523 mg. The calcium in the diet of Group II was mostly obtained from vegetable sources and hence a large amount of it was in the form of phytin.

The maximum, minimum and average data for adults for the daily intake of phosphorus are 2,201 mg, 1,865 mg, and 2,034 mg in Group I, and 1,979 mg, 1,115 mg, and 1,448 mg in Group II. The phosphorus intake of the beggars was quite low.

From the above observations the following points emerge:

(1) Clinically, the physical status of the two groups did not vary much.

(2) The weights of the beggars were consistently lower in relation to age, sex and height and also in comparison to those of medical students, which in turn were less than the western standards.

(3) The diet of both groups lacked protective foods. The supply of calories was highly insufficient. The intakes of animal proteins, vitamins A, B (thiamine, riboflavin and niacin) C and D and minerals (Fe Ca and P) was very low, especially in the beggar group.

(4) No radiologic abnormality was detected in the architecture or chemical organiza-

TABLE II

Chemical Composition of Diet per Person per Day

Subjects	Total calories	Total proteins *g*	Animal proteins *g*	Vitamin A I.U.	Thiamine *mg*	Riboflavin *mg*	Niacin *mg*	Vitamin C *mg* 1 *mg* = 20 I.U.	Vitamin D I.U.	Iron *mg*	Calcium *mg*	Phosphorus *mg*
Group I												
Adults	2,340	86	13	184	0.46	0.34	6	22.4	6	10	928	2,034
Group II												
Adults	1,744	65	1	253	1.04	0.63	9	1.4	1	12	276	1,448
Pregnant women	1,620	58	4	168	1.08	0.74	11	0.2	2	12	319	1,244
Lactating women	2,338	85	5	288	1.31	0.74	12	0.4	2	14	436	1,687
Children	1,260	36	8	102	0.57	0.35	6	1.3	1	6	173	809

tion of the skeleton of clinically healthy persons.

(5) Among the beggars the incidence of vitamins A, B, C and D deficiency was 2.5 per cent, 1.25 per cent, 0 per cent and 0.62 per cent respectively; 93.75 per cent of the beggars were free from symptons and signs of avitaminosis or hypovitaminosis.

DISCUSSION

Although the technic adopted for the assessment of the dietary suffers from the usual defects of such a procedure, an improvement has been achieved in the present study by taking into consideration a wide variation in the consumption of food materials for a long period of time, spread over several years.

Utmost care was taken to ensure accuracy of records and all aspects have been duly considered in calculating the dietary intakes and in computing the chemical values of foodstuffs. The subjects were told in advance of the purpose for which the data were being collected and were individually requested not to hide or exaggerate anything. Because of the peculiar mode of life of the beggars no other alternative direct method of assessing the diet was available and it is the considered opinion of the author that the calculated dietary intakes are very close to reality.

The maximum eating capacity determinations indicated that the beggars were always underfed. They ate more if and when more food was offered them.

The average daily intake for several years has been derived from present intakes after considering a great many variable, i.e., fluctuation in the prices, purchasing capacity and available types and amounts of foodstuffs which could be obtained as "alms."

There is, therefore, much justification in regarding these data as fairly indicative of the state of nutritional intakes in spite of the fact that the technic which had to be followed in obtaining them cannot be claimed to be flawless.

The general character of the diet of Group II represents considerable inadequacy in the amount of growth-promoting and bone-building dietary essentials available for daily consumption, in addition to its being grossly unbalanced and of low caloric value.

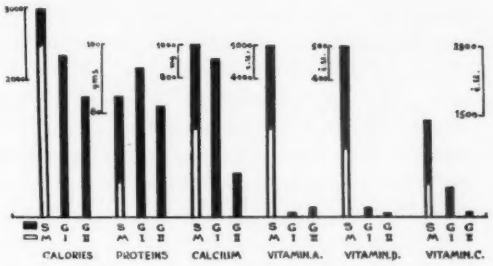

Fig. 2. Comparison of daily requirements and actual intakes of calories, proteins, calcium and vitamins A, D, C. S = Standard daily requirements. M = Minimum daily requirement. Other notation same as in Figure 1.

Although height and to some extent weight are influenced also by extra-dietary factors, the need for proteins of high biologic value for development and growth of infants and children[5–8] and maintenance of adults[9–13] is imperative. Pregnancy and lactation[14–16] and illness[17,18] require special provisions. The recommended daily allowance of proteins for adults is 1 g/kg and for children it is 2 g/kg, after the age of three years. Considering these recommendations, it is found that the average protein intake in relation to the average weight of adults of Groups I and II is about 37.2 per cent and 51.1 per cent more. The intake levels of proteins of high biologic value are, however, surprisingly low and this may be partly related to the low heights and weights.

The vitamin content of the diets of both the groups is less than the minimum requirements. If the losses due to processing and in absorption are taken into account one would wonder if anything is actually available at all.

A comparison of the recommended standard daily allowances, the minimum requirements, and the actual dietary intakes of adult males of the two groups for proteins, calcium, and vitamins A, D and C is presented in Figure 2. from which the gross inadequacy of these vitamins in the diets, especially of Group II, is apparent.

It is, however, surprising that even such low intake levels were not associated with signs and symptoms of deficiency states. The occurrence of normal calcification of the skeleton

in the face of very low intakes of vitamin D may be explicable on the basis of the protective effects of the strong intensity of ultraviolet light. Similarly, an increased synthesis of vitamin B factors by the intestinal flora, can be presumed. But the mechanism of compensation for the low intakes of vitamin A and C is difficult to imagine. It can only be presumed that some internal adjustments might have taken place which reduced the necessary requirements even below the minimum level hitherto believed possible.

It is significant to note that in the diet of Group II individuals, the low intake of animal proteins, and vitamin A,B,C, and D was associated with a low-calcium intake. Many workers have come to the conclusion after carefully conducted studies, that the utilization of calcium is influenced by proteins and vitamins.[19–21]

Rose,[22] Rose and MacLeod,[23] Fincke and Sherman,[24] Tisdall and Drake,[25] and Spiers[26] have carried out extensive work on the availability of calcium from different sources. Calcium of the cereals is mainly in the form of phytin-calcium-magnesium salt of inositol phosphoric acid.[27] Not only is the calcium of the phytin difficult to absorb but it also interferes with the absorption of calcium taken in other forms. An anticalcifying effect of the cereals has also been suggested by Krebs and Mellanby.[28] It seems, however, necessary to presume that some calcium must be absorbed even in those who subsisted mainly on cereals, because the subjects of Group II did have a normally mineralized skeleton.

Steggerda and Mitchell[29] and Outhouse and co-workers[30] have placed the daily maintenance requirement of calcium between 0.67 to 0.75 g per day. Similar observations have been made by Chu[31] on Chinese subjects. The requirements for children and pregnant and lactating females have been calculated to be much higher.[32–35] There is a marked increase in urinary excretion of calcium in late pregnancy and lactation.[25,32]

Taking into account the minimum daily requirements and the amount and form of calcium available to the children and adolescents of Group II, it appears that with a calcium intake of less than one-fifth of normal along with a diet deficient in vitamins A, C, D and animal proteins which have been established to be highly important for calcium retention, the children of this group must take about five times the duration of normal growth period. This, however, is not borne out by observations on the present series where the mineralization of skeleton has been found to progress satisfactorily; there being no clinical or radiologic evidence of calcium deficiency. The calcium intakes during pregnancy and lactation were surprisingly low without any evidence of deficiency.

Several other workers have found low intakes of calcium in different countries with or without incidence of deficiency.[36,37] If calcium deficiency in the diet cannot be correlated with the incidence of deficiency states in the tropics, one has to assume that the low intake of calcium over a period of generations has brought about some physiologic adjustments which help to economize this essential factor.

The occurrence of growth in infancy and childhood, almost normal mineralization of skeleton and maintenance of normally calcified skeleton during adult life and even during pregnancy, lactation, and illness, with very low intakes of calcium, indicate some type of adaptation of low intakes. In the case of calcium this low intake adaptation may function by maintaining a high net absorption aided by a rigid internal economy and rectification of the anticalcifying effect of cereals by strong ultraviolet light of the tropics.[38]

CONCLUSION

In consequence of a long continued inadequate diet providing low intakes of calories, animal proteins, vitamins and minerals the body seems to develop a "nutritional adaptation" to low intakes. Such an adaptation may function through increased absorption and selective internal economy favoring more important needs (growth and mineralization of skeleton, vital metabolic needs) rather than the less important ones (body weight).

SUMMARY

The assessment of the diet of 240 subjects selected from two socio-economic groups (med-

ical students and street beggars) indicates a continued low intake of total calories, animal proteins, vitamins (A, B, C, and D) and mineral (iron, calcium, phosphorus) especially in the beggars, for several years.

Clinical examination did not indicate the presence of nutritional deficiency states in 93.75 per cent of the beggars. The nutritional status of the beggar group was, however, inferior to that of the medical students in respect to body weight.

Radiologic examination indicated a normal calcified skeleton in clinically healthy subjects.

These low intakes apparently did not seem to influence very much the course of development and growth in children, or of pregnancy and lactation in women of the beggar group.

It has been concluded that in consequence of a prolonged low intake the body seems to develop some sort of "nutritional adaptation" so that development, growth, and maintenance even in the face of the extra demands of pregnancy, lactation, or illness, are possible with quite low intakes. The "nutritional adaptation" may function by exercising a very rigid internal economy in the interest of more important functions.

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Calculi and Kidney Calcification from Feeding Milk Diets to Rats and Hamsters

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FOR CENTURIES men have sought methods to prevent the formation of calculi in the kidneys and bladder. Much effort has also been devoted to discover methods of dissolving calculi after they are formed.

If more rapid progress is to be made in this field, better use must be made of suitable test animals. The rat and the Syrian hamster seemed worthy of study to determine if they might be useful for such research.

During the past two decades, the calcification of the kidneys of rats has been given some attention in our laboratory, especially by Barnes,[1] Sperling *et al.*,[2] and Saxton *et al.*[3] The kidneys of female rats have proved to be more vulnerable to calcification than those of the males. Diets composed chiefly of whole milk supplemented with trace minerals have proved especially effective in producing calcification of the female kidneys. In the course of these studies numerous observations have been made of calculi in the bladders of white rats dying in old age.

Numerous reports in clinical medicine attempt to relate calculi to the long continued use of Sippy diets. These diets, formerly much used in ulcer treatment, involved the ingestion of substantial amounts of milk and sodium bicarbonate. In a modern text such as that of Ivy *et al.*[4] summaries are given which indicate that the evidence is not entirely convincing that milk and bicarbonate may lead to an increased incidence of calculi.

Since milk itself tends to produce calculi in rats, one may ask if this is related to the high calcium phosphate level provided by milk, to the influence of milk diets upon the pH of the urine or to the low magnesium content of milk. Many studies have implicated magnesium in the formation of calculi,[5] but no consideration was given to it in these experiments.

Although little attention has been given to hamsters in the course of long-term experiments, this species might seem more useful than the rat for such studies because it excretes much more calcium in the urine than does the rat. Hence, one might expect the kidney of the hamster to suffer from deposition of calcium. However, the hamster is much less efficient than the rat in the absorption of calcium from the intestinal tract.[6] It is also more difficult to feed liquid milk diets to the hamster since it tends to ingest less fluid.

The present studies were undertaken to extend earlier observations with rats and to compare the effects of feeding milk to the two species. Attempts were also made to determine the time needed to produce calcification of the kidneys. Since some claims have been made by clinicians that calcification can be reversed in patients, an initial attempt was made to change diets and determine if calcification could be reversed.

Earlier studies had indicated that milk diets tend to protect teeth against decay, help maintain denser bones in old age, and often lead to the formation of balls of hair (trichobezoars) in the stomach of rats. Therefore, attention was paid to these problems in the course of these experiments.

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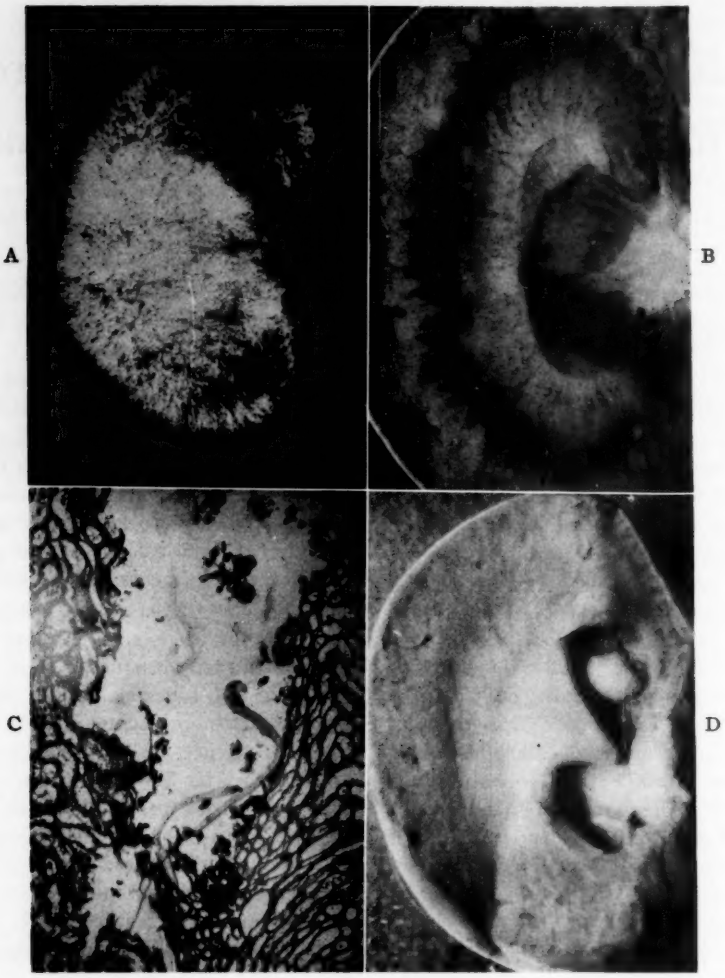

Fig. 1. A. Calcification in medulla of kidney of female rat as shown by x-ray. B. Calcification in medulla of kidney of female rat shown by von Kossa's silver nitrate stain. Small calculi also are present in pelvis. C. Calculi of microscopic size in calyx of male rat's kidney. D. Large calculus in pelvis of kidney of male rat. Small calculi are seen behind the large one.

(*A*) *First Study with Rats and Hamsters*

Thirty weanling rats were divided into three groups of ten each, with the sexes evenly divided. The diet consisted only of fresh, pasteurized, homogenized, vitamin D-milk supplemented with the following trace elements/100 ml: Mn 1.5 mg as $MnCl_2$, Cu 0.8 mg as $CuSO_4$, Fe 5.0 mg as $FeCl_2$ and I 2 mg as KI. The milk had the usual addition of 400 units of vitamin D per quart.

The three groups of rats were fed the following diets: (1) milk; (2) milk containing 0.25 per cent $NaHCO_3$; (3) milk and bicarbonate with an additional daily allowance of 4 g of a solid mixture consisting of 1 g of finely ground cellophane mixed with 3 g of sucrose.

In order to determine the number of months needed to produce calcification in the kidneys of females, one rat in each group of this sex was killed at two, four, and eight months after

weaning. After eight months the calcification was detected readily by staining with either eosin-hematoxylin or silver nitrate (von Kossa).

After 12 months of feeding the diets, two male rats from each group were killed. Four rats from group 1 and 3 from group 2 were then fed a complete stock diet until 16 months from the start of the experiment. All were then killed.

The degree of calcification of the kidneys was determined by three different methods: roentgenograms, gross observation and microscopic study of sections. The bladders were examined for calculi (Fig. 1).

Calcification was found in the medulla of the kidneys of the females with no evidence that this had been alleviated by stopping the milk diet after 12 months. Calcification was found in the pelvis of the kidneys of both sexes. More calcification seemed to occur in animals that had been fed bicarbonate in the milk. Calculi were found in the bladders of one female and four males with no relation to groups.

Dental caries were observed only in group 3 which had been fed sugar and cellophane. No hair-balls were found in the stomachs of the rats of this group but two instances were found in the rats in group 1 which were fed milk only.

An experiment with 30 hamsters similar to that with rats was started at the same time. Due to external circumstances, this experiment could not be completed, but did yield some useful information. At the start of the study the trace elements were not added to the milk. After two months the hemoglobin concentration of the hamsters had dropped to 60 per cent of the normal and the hair of the animals turned gray. Soon after addition of trace elements the hemoglobin level became normal and the fur returned to its golden color. However, the animals died in the course of a few months until only three survived in group 1, none in group 2 and three in group 3 at 11 months after the start.

Only one hamster in group 3 showed very slight calcification in the kidneys. Two of the three animals from group 1 had hair-balls in the stomach while none of the three fed cellophane had these. One female from the group 3 fed sucrose had carious teeth.

(*B*) *Second Study Using Hamsters*

Twenty-four hamsters were divided into two groups, with the sexes evenly distributed. The first group was fed mineralized milk like those in the first study with 0.25 per cent of sodium bicarbonate dissolved in the milk. The second group was given the milk, but 10 per cent sucrose was dissolved in it. Bicarbonate was added to this milk at a level so that the ingestion of the bicarbonate was equal to that of the animals of group 1. The animals of group 2 drank less instead of more of the sweetened milk.

At the end of six months there were only eight hamsters alive in group 1 and four in group 2. These were all killed by etherizing, for study. No calculi were found in the bladder. Very slight calcification could be detected in the pelvis of 40 per cent of the animals. The kidneys of the hamsters were free of casts and the degenerative changes commonly observed in the kidneys of rats. Five of the eight animals in group 1 and one of the four in group 2 had hair-balls in the stomach. Only one animal, in group 2, had carious teeth.

These exploratory studies indicated that the hamster was very resistant to kidney changes while the rat was subject to them.

(*C*) *Third Study Using Rats and Hamsters*

Thirty weanling rats were divided into three groups with the sexes evenly distributed. They were fed as follows: Group 1: fresh mineralized milk; group 2: fresh mineralized milk containing 0.25 per cent $NaHCO_3$; group 3: same as group 2 except that the milk contained 10 per cent sucrose.

Since the males drank more milk than the females and the animals in group 3 drank less milk than the others, the bicarbonate was equalized to that consumed by the females of group 2.

At the end of six months all rats were killed. In Table I the milk consumption per 100 g of live body weight is given for a series of representative weeks. On the basis of body weight the females consume more milk than the males. This has been observed previously in other experiments.

TABLE I

Milk Consumption in cc/100 g of Body Weight/Day. Mean Milk Intake with Standard Deviation (See Text, Section C)

Time on Diet	4th week	8th week	12th week	16th week	20th week
Rat					
Group 1 Milk					
F	56 ± 1.7	40 ± 2.0	34 ± 1.4	30 ± 0.6	28 ± 1.1
M	56 ± 4.0	39 ± 2.7	31 ± 2.6	27 ± 0.6	23 ± 2.3
Group 2 Milk and $NaHCO_3$ 0.25%					
F	57 ± 1.9	42 ± 2.8	38 ± 1.7	34 ± 2.1	32 ± 1.3
M	55 ± 5.3	39 ± 3.3	32 ± 2.7	30 ± 3.4	26 ± 1.6
Group 3 Milk, $NaHCO_3$ 0.25% and sucrose 10% dissolved in it					
F	46 ± 4.3	31 ± 3.7	29 ± 2.0	25 ± 3.9	23 ± 3.6
	46 ± 2.3	31 ± 1.5	27 ± 2.5	21 ± 1.1	21 ± 2.8
Hamster					
Group 1 Milk					
F*	49 ± 0.0	47 ± 0.0	39 ± 0.0	41 ± 0.0	...
M	48 ± 1.3	45 ± 4.3	31 ± 11.6	36 ± 10.0	...
Group 2 Milk and $NaHCO_3$ 0.25%					
F	53 ± 3.0	53 ± 4.0	48 ± 3.0	36 ± 13.0	...
	48 ± 1.0	49 ± 5.0	41 ± 4.0	42 ± 1.0	...
Group 3 Milk, $NaHCO_3$ 0.25% and sucrose 10% dissolved in it					
F	31 ± 3.0	35 ± 3.0	29 ± 3.0	33 ± 2.0	...
M	29 ± 2.9	30 ± 2.1	26 ± 1.8	24 ± 3.8	...

* Only one animal was left in this group; no standard deviation was obtained.

F = female; M = male.

The same sex differences were found as in the first study. Calcification was found in the medullas of the kidneys of the females but not in those of the males. In five of the females given bicarbonate, the calcification was also marked in the pelvis. Calcification seemed more marked especially from the x-ray evidence in animals given bicarbonate and seemed somewhat worse in group 3 which received sucrose in the milk.

Calculi were found only in the bladders of males. Ten of the fifteen males had these calculi with even distribution among the three groups. The extra case was in group 2. No females had calculi in the bladder.

No carious teeth were observed and only one hair-ball.

Thirty-six weanling hamsters were divided into three groups and given the same diets as the rats. Only 14 hamsters survived for five months when they were killed. No calculi were found in the bladders of any animal. Only very slight evidence of calcification was found in the pelvis of the kidneys of 7 hamsters. Two of the animals had hair-balls and none had carious teeth.

DISCUSSION

From these studies it seems that the rat is an excellent animal for use in the study of calcium deposits and calculi. The female is more subject to calcium deposits and the male to calculi. The hamster is not a satisfactory animal for such research because its survival is poor on milk diets. Furthermore, its kidneys seem much more resistant to deterioration including calcification than are those of the white rat. In such studies it is evident that both

species suffer from hair-balls when given a diet of liquid milk unless some additional source of roughage is made available.

Although there was no evidence of a reversal of calcification by changing the diet in a single attempt, it seems that rats fed diets rich in liquid milk may afford a useful method for devising methods of reversing calcification of the kidneys and dissolving calculi in the bladder.

SUMMARY

Three studies in which rats and hamsters were fed diets composed of fresh milk supplemented with trace minerals, sodium bicarbonate, and in some cases, sucrose, indicate that the female rat is very subject to calcification of the kidneys while the male suffers from calculi of the bladder. The Syrian hamster is difficult to maintain on fresh milk diets but develops neither calcium deposits in the kidneys nor calculi in the bladder. No evidence was found that these deposits could be dissolved after rats were changed to another diet for three months. The oldest rats were continued on the diets for a year while the hamsters in the longest study were fed the milk for 11 months. The white rat seems to be a very useful species for the study of the effects of Sippy diets and methods of reversing calcification of kidney tissues and calculi of the bladder.

To prevent complications from hair-balls in the stomachs of experimental animals, it seems useful in studies of liquid milk diets to feed a gram of cellulose per day to each animal. This can be mixed with sucrose and fed separately but such animals suffer from decay of the teeth. In neither rats nor hamsters are the teeth affected if sucrose is dissolved in the milk and not fed separately.

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Serum Cholinesterase Levels of Central American Children in Relation to Nutritional Status

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PRIOR to 1948 limited information was available to suggest a direct relationship between cholinesterase serum levels and nutritional status.[1-3] More recently, McCance *et al.*,[4] and Hutchinson and co-workers[5] reporting on the Wuppertal Studies, have demonstrated a significant decrease in serum cholinesterase activity with severe undernutrition. With re-feeding, the values increased toward, and even above, normal. Studies of children with kwashiorkor[6-8] indicate that serum cholinesterase activity is low and rises with treatment parallel to the clinical improvement. There are, however, no reports as to whether cholinesterase activity decreases before clinical signs of kwashiorkor develop.

Investigation of nutritional deficiency diseases in Central America[9] has indicated that the incidence of kwashiorkor, a severe form of protein malnutrition, is greatest between the ages of one and four years and that moderate protein deficiency (prekwashiorkor) is widespread in this age group.[10,11] If serum cholinesterase activity were a sensitive measure of protein malnutrition, low values should be found often among poorly nourished children of this age. In order to investigate this possibility, serum cholinesterase activity was measured in groups of children differing markedly in nutritional and socio-economic background. Children of both preschool (one to six years) and school age (seven to twelve years) were included in the study.

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ECONOMIC AND SOCIAL STATUS OF THE GROUPS IN SURVEY

School Children

Four groups of school children aged seven to twelve years were examined. Those from the Guatemalan schools served simultaneously as subjects for a study of serum cholesterol levels.[12] The Nicaraguan children were part of a clinical nutrition survey of families in a poor suburb of Managua.[13]

Group I, Guatemala Upper Income Urban: The school children in this group came from relatively well-to-do families.* Their fathers were business and professional men in Guatemala City. Only children having at least one Guatemalan parent were included.

Group II, Guatemala Lower Income Urban: These children came from families who lived in a relatively poor district of Guatemala City. Their fathers worked as mailmen, policemen, truck and bus drivers, or as unskilled or semiskilled laborers.

Group III, Nicaragua Lower Income Urban: The children included in this group had very similar socio-economic backgrounds to those included in Group II, but their families lived in a relatively poor district of Managua, Nicaragua.

Group IV, Guatemala Lower Income Rural: The families of the children included in this

* The cooperation of Mr. Robert B. MacVean, Director of the "Colegio Americano de Guatemala" made possible the collection of these samples.

group lived in and around the rural village of San Miguel Dueñas, located about 35 miles from Guatemala City. The fathers of most of these children were agricultural laborers on nearby farms and coffee plantations or cultivated their own small plots of land.

Preschool Children

Central American children of both sexes from one to six years of age were chosen as follows:

Group V, Guatemala Upper Income Urban: The preschool children in this group came from families with the same favorable socio-economic status as those of the school children of Group I. All were attending a private nursery school in Guatemala City.*

Group VI, El Salvador Lower Income Urban: The parents of the children included in this group earned very low salaries working as unskilled or semiskilled laborers. They lived in a very poor district of San Salvador, El Salvador.

Group VII, Nicaragua Lower Income Urban: In this group were included the children from the families of Group III who were not yet attending school.

Groups VIII and IX, Guatemala Lower Income Urban: The parents of the preschool children of these groups lived on two coffee plantations in Guatemala and worked as agricultural laborers. They had a very low rural standard of living.

Group X, Kwashiorkor: The children in this group were suffering from severe protein malnutrition (kwashiorkor), and were sampled on the day of admission to the hospital.

Dietary Status

No precise individual dietary information was available for most of the children studied, but the diets of their parents, or of adults of comparable socio-economic groups, have been determined by daily dietary interviews for periods of seven days. The total intake of calories, protein and fat, as well as the proportions of animal and vegetable protein and fat are summarized in Table I.

These data indicate basic differences in the dietary patterns characteristic of the socio-economic levels, as exemplified by the daily intake of calories, total protein and fat, and particularly by the amounts of the two latter nutrients of vegetable or animal origin. They also illustrate the close correspondence between the proportion of the total dietary proteins which are of animal origin and the economic and social status.

METHODS

Approximately eight children of each sex were selected at random for each year of age from seven through twelve, in each of the four school-age groups. Preschool children were also studied in each year of age from one through six. About 0.1 ml of blood was obtained by a fingertip puncture and the serum promptly separated and frozen. The samples were analyzed within two weeks after collection.

The total cholinesterase activity was determined by a micro-adaptation of the method of Reinhold *et al.*[19] using 0.02 ml aliquots

* The cooperation of Mrs. Evelyn Rogers, Director of the School is gratefully acknowledged.

TABLE I

Characteristic Dietary Estimates of Adults of Different Socio-Economic Status

Socio-economic status	Calories	Protein g/day			Fat g/day		
		Animal	Vegetable	Total	Animal	Vegetable	Total
Guatemala upper income urban[14]	2,462	39	30	69	46	56	102
Guatemala lower income urban[15]	1,585	10	37	47	13	14	27
Nicaragua lower income urban[16]	1,928	26	36	62	—	—	53
El Salvador lower income urban[17]	1,784	25	35	60	—	—	48
Guatemala lower income rural[18]	2,283	6	61	67	9	14	23

of serum. The results are expressed in Michel Units.[20] Determinations were run in duplicate, using 0.01 per cent phenol red as the indicator of pH change.

Replicate aliquots of a quantity of pooled sera from normal adults were analyzed with each group of samples studied. The average value of 15 independent determinations in the pooled specimen was 1.11 ± 0.08 Michel Units. This value, as well as the averages for the groups studied, is higher than previously reported values for normal adults obtained by the original Reinhold *et al.*[19] method. The discrepancy may be explained by the fact that in the modified technic employed in the present study, the tubes were allowed to incubate only until the indicator showed that they had reached a pH of about 7, instead of incubating them for a fixed period of one hour. The modification introduced results in an improvement of the method, since it gives a better measure of the initial velocity of the reaction, which is in turn proportional to the enzyme concentration. With samples of normal activity the pH decreases in one hour to a point where the optical density change is very small per unit of pH change, and under these conditions the method becomes insensitive. In addition, it is known that the activity drops with decreasing pH.

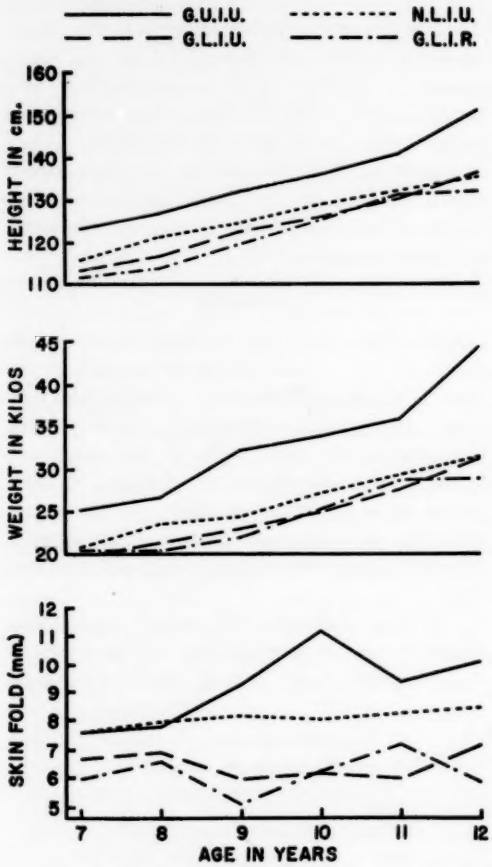

Fig. 1. Physical measurements of the experimental groups of school children. G.U.I.U., Guatemala Upper Income Urban (Group I); N.L.I.U., Nicaragua Lower Income Urban (Group III); G.L.I.U., Guatemala Lower Income Urban (Group II); G.L.I.R., Guatemala Lower Income Rural (Group IV).

Height, weight, and skin-fold thickness of the children were determined at the time of taking the blood samples. The measurements of the thickness of the skin-fold of the posterior surface of the upper arm were made as recommended by Brožek *et al.*[21]

RESULTS

The height, weight, and skin-fold thickness for the four groups of school-age children are portrayed graphically in Figure 1 and illustrate the differences in these measurements among the different socio-economic groups. It should be noticed that in general the "upper income urban" children are taller and heavier and have more subcutaneous fat than the other urban and rural groups; the latter show the lowest figures for the nutritional status indices employed.

The serum cholinesterase activity values are summarized in Table II. The standard deviations and the standard errors were similar in the different groups. Even though some differences occur among them in the average cholinesterase activity levels, no tendency of the enzyme levels to be related to the socio-economic standard or to the nutritional status of the children is suggested by the data. The marked decrease in serum cholinesterase activity characteristic of the severe protein malnutrition of kwashiorkor is illustrated in Table II by the low average value for 18 cases.

TABLE II

Serum Cholinesterase Values in Children of Different Socio-Economic Status

No. of group	Group description	Number of children	Serum cholinesterase (Michel Units ΔpH/hr	
			Mean ± S.E.	S.D.
	School children:			
I	Guatemala upper income urban	88	1.12 ± 0.02	0.19
II	Guatemala lower income urban	88	1.02 ± 0.02	0.17
III	Nicaragua lower income urban	35	1.03 ± 0.04	0.23
IV	Guatemala lower income rural	89	1.18 ± 0.02	0.23
	Preschool children:			
V	Guatemala upper income urban	19	1.28 ± 0.05	0.23
VI	El Salvador lower income urban	39	1.20 ± 0.04	0.27
VII	Nicaragua lower income urban	42	1.10 ± 0.03	0.19
VIII	Guatemala lower income rural	30	1.21 ± 0.06	0.32
IX	Guatemala lower income rural	48	1.00 ± 0.02	0.16
X	Kwashiorkor	18	0.30 ± 0.04	0.15

DISCUSSION

In view of the finding that the levels of serum cholinesterase are markedly reduced in severe protein malnutrition[6–8] (see Table II) it was not unreasonable to expect that low values might be found among lower income rural children, since in these groups kwashiorkor or the clinical stage just preceding the syndrome (prekwashiorkor) is common.[10] The fact that the values encountered were not low strongly suggests that the decrease in cholinesterase to the low levels found in kwashiorkor is a relatively sharp fall rather than a gradual one, and that it probably occurs only after clinical signs of protein malnutrition have begun to develop.

The data presented indicate that serum cholinesterase levels are not a sensitive means for evaluating nutritional status, since the large differences observed in this study between the upper and lower income groups, both in urban and rural areas, were not reflected in consistent differences in enzyme levels. On the other hand, the physical measurements of height, weight, and skin-fold thickness did show a close, direct relationship to the adequacy of the dietary patterns. Therefore, it is reasonable to consider them a more sensitive measure of mild to moderate nutritional deficiency than serum cholinesterase activity. This is the same conclusion reached by Saunders *et al.*[22] in studies of United States high school children where, however, the range of malnutrition was not nearly so great.

SUMMARY

The levels of serum cholinesterase activity were studied in four groups of school children (age seven to twelve) and in five groups of preschool children (age one to six) from different socio-economic and nutritional backgrounds. The following values for school-age children were found: Guatemala upper income urban, 1.12 ± 0.02; Guatemala lower income urban, 1.02 ± 0.02; Nicaragua lower income urban, 1.03 ± 0.04; and Guatemala lower income rural 1.18 ± 0.02. For preschool children the levels obtained were: Guatemala upper income urban, 1.28 ± 0.05; El Salvador lower income urban, 1.20 ± 0.04; Nicaragua lower income urban, 1.10 ± 0.03; Guatemala lower income rural, first group, 1.21 ± 0.06, and second group 1.00 ± 0.02. The cholinesterase activity showed no tendency to be related to the socio-economic background or the nutritional status of these children. Eighteen children age one to five with the severe protein deficiency of kwashiorkor showed a serum cholinesterase value of 0.30 ± 0.04. It is concluded that serum cholinesterase is not a sensitive measure of protein deficiency and drops only when the deficiency is so severe as to be clinically apparent.

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Editorial

Dietary Fat and Human Health: Current Recommendations

Interest in the possible role of dietary fat in atherogenesis has served as a stimulus to intensive research in lipid metabolism. This focus on lipids has brought an awareness of the paucity of information concerning the role of fat in nutrition and health. Recent research has uncovered many interesting findings but data are far from complete and are often conflicting. Misconceptions are common and hypotheses and opinions are frequently confused with facts.

Dietary fat has not been proved to be etiologically related to atherosclerosis and coronary artery disease. Available evidence suggests rather that disorders of fat transport and metabolism may have an important role in the atherosclerotic process. However, there seems little doubt that multiple factors influence atherogenesis including heredity, mechanical and hormonal factors, possibly nutrients other than fat and disorders of the blood clotting mechanism.

Studies in experimental animals and in man have shown that, among other factors, the amount and type of dietary fat can influence serum lipid levels. An association between elevated serum lipid concentrations and atherosclerosis has also been observed. However, that reduction of serum lipids will prevent or reverse atheromatous changes in human blood vessels remains to be demonstrated. The mechanisms by which serum lipid concentrations change in response to variations in dietary fat remain largely unknown.

We do not know the amount or the types of fat which should be included in the diet to insure good nutrition and health. Recent research makes it obvious that fat can no longer be considered as an entity and that attention must be paid to specific lipids and their components, i.e., fatty acids (including chain length, degree of saturation and isomerization), sterols, phospholipids and even minor constituents of the triglycerides which constitute the major source of dietary fat. Certain polyunsaturated fatty acids, linoleic, linolenic and arachidonic, have been found to be dietary essentials in many animal species. Presumably these acids are essential in man but such has not been demonstrated unequivocally. The metabolic role of the essential fatty acids has not been elucidated although some evidence suggests a function in the transport of fat. This has led to the hypothesis that essential fatty acid deficiency may play a part in the pathogenesis of atherosclerosis. Much investigation will be required to prove or disprove this interesting theory.

In spite of the many unknowns, the physician has the responsibility of advising his patients as to diet, including the quantity and kinds of fat to be included. Certainly some fat is indicated. Fat is a concentrated source of energy, exerts a protein-sparing action, contributes palatability and satiety to the diet, and assists in the utilization of the fat-soluble vitamins, which are also supplied by certain fats.

In view of the limitations of present knowledge, it seems unwise to suggest any major change in the dietary pattern of healthy persons. A good, varied diet that avoids caloric excess should be recommended as the basis for good nutrition. In the control of obesity, some restriction of fat intake is usually indicated in view of the high caloric value of fat. In diseases in which hyperlipemia is a feature, dietary therapy should be directed toward lowering lipid levels. In patients with coronary artery disease or with other manifestations of atherosclerosis,

it may be desirable to limit total fat intake or to increase the intake of unsaturated fats as an experimental therapeutic procedure. Such therapy should be followed and evaluated carefully, using both clinical and laboratory observations. Much can be learned by therapeutic studies that are well controlled and continued for long periods of time.

Encouragement of basic and clinical research in all phases of lipid metabolism is highly desirable so that answers to many unsolved problems can be obtained and the role of dietary fat in human health can be delineated with precision.

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Once Upon a Time

"Once upon a time there was a very poor country, where nobody had enough to eat and the average expectation of life was 24 years. There was also a very rich country, where everybody had plenty to eat and the average expectation of life was 64 years. In the very rich country people used to save up milk and butter and cream and eggs and send them to the very poor country, where they were distributed, especially to the children, who would otherwise have had none. In this way the expectation of life in the very poor country was raised from 24 to 27 years. Meanwhile the expectation of life in the very rich country was rising too, and went up from 64 to 67 years, and everyone who didn't die of cancer of the lung from smoking too many cigarettes died of coronary thrombosis. Then someone discovered that coronary thrombosis was due to eating and drinking too much milk and butter and cream and eggs in the very rich country, and sent it all to the very poor country, so that the expectation of life in the very poor country might be raised high enough for them to start dying of coronary thrombosis so that they, too, could stop eating and drinking milk and butter and cream and eggs."

—A Running Commentary by Peripatetic Correspondents. *Lancet* 18: 635, 1956.

Letters to the Editor

On the Relationship between Diet and Arteriosclerosis

Dear Sir:

In a recent Editorial of your Journal (5:674, 1957), Dr. William Dock raises the question: "How will the medical historian of the year 2207 regard the reaction of some segments of the medical profession in the last half century to the vast and concordant evidence for the dietary basis of atherosclerosis?"

Belonging to the more skeptical "segment," I freely admit that there are many still controversial data published in the literature which indicate that dietary factors may possibly modify the course of atherosclerosis. In spite of the vast literature on this problem, however, no evidence has yet been provided which *proves* that human atherosclerosis is produced by the consumption of fat or, particularly, butter fat, as suggested in the Editorial by Dr. Dock. It seems to be logical, therefore, that a large segment of investigators is waiting for more conclusive evidences before accepting any causal connection between diet and atherosclerosis. We just have to take our chances with the historian of the year 2207! But did Dr. Dock consider what Clio's judgment may be of physicians who, not so long ago, accepted as truth the theory based on statistical coincidence, that malaria was produced by "mala aria," i.e., by "bad air" or that excessive sugar consumption is responsible for diabetes?

—Ernest Geiger, M.D., Ph.D.
University of Southern California
Los Angeles, California

Comment by Dr. Dock

Dear Sir:

The doctors who believed malaria was due to "vapors" made fun of the work of Laveran, and of those who worked out the cycle of malarial plasmodia in crows and canaries, just as those who believe arteriosclerosis is due to degeneration make fun of experiments on chicks, rabbits, dogs or monkeys. In these young animals dietary manipulation, and dietary manipulation alone, raises blood cholesterol (as does dietary manipulation in man) and produces atherosclerosis, not seen in controls or much older normal animals. In rats, rabbits, chicks and monkeys, butter fat does raise blood cholesterol and in rats causes coronary disease and myocardial infarction not seen in controls on corn oil. Since people who use no butter fat, in America, Asia and Africa, are remarkably free of atherosclerosis, and have blood cholesterol levels under 180 mg/100 ml it seems to me that those who are skeptical share the mental attitude of those who rejected the avian work in malaria, or Harvey's work on the circulation. Those who reject animal evidence concordant with clinical experience should be rabid antivivisectionists, for animals should not be used if the facts they reveal do not apply to man.

The point I tried to make was that since 1915 the evidence that diet rich in animal fat was the *sine qua non* for atherosclerosis has been far more compelling than evidence for the circulation was in 1628 or even 1678.

—William Dock, M.D.
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Diet Therapy

The Physician's Role in the School Lunch Program

JESSIE CRAIG OBERT, PH.D.*

SCHOOL lunch programs provide one of the best potential channels for the promotion of better health, the inculcation of good food habits, and the improvement of conditions for learning. In our democratic society it is a responsibility of community leaders to support and interpret programs which help to improve the overall level of health in the community. The school lunch program needs the understanding and support of physicians and other leading citizens if its maximum possibilities for improving nutritional status and developing good food habits are to be realized. Support of the physician is of particular importance because of his role in individual and community health.

Let us picture for a moment the noon meal in a school where the lunchroom is a dynamic part of the educational program. The lunchroom is decorated with murals made by art students and there is soft music from records selected by a music class. Inspection of lunchroom and kitchen presents evidence of good sanitation practices.

The children have washed their hands before coming to the lunchroom and politely, though perhaps a bit impatiently, wait in line, for after all they are healthy, well-nourished, lively children. Some have studied about a new vegetable and look forward to their first taste, while others have helped plan the meal. All have eaten a good lunch together every day during the school year and look upon this as a regular part of the day's activities. The delicious food is served quickly in attractive fashion by pleasant, friendly cooks under the supervision of a lunchroom manager whose training and status are similar to those of the teachers.

A few children whose families cannot pay for their lunches, receive free meals but only the teacher knows who they are. Children who have brought box lunches purchase milk or other foods and eat in the lunchroom with their classmates. All take their places at the tables where they eat with their friends under the supervision of teachers or older boys and girls. Most of them eat all of the foods served, so there is little food left on the plates.

Scenes like the one just described should occur in many more schools. It is the purpose of this paper to examine the objectives of the school lunch program, to consider its present extent and the effect of the National School Lunch Act, to discuss the "Type A" lunch, to consider the role of various members of the school personnel, to mention some of the problems, and finally, to suggest a few ways in which the physician in a community can support this important program for improving the health of the nation's children.

WHY THE SCHOOL LUNCH?

To most persons the lunch, is, first of all, a feeding program. Under the usual school regime the child is away from home most of the

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time between the morning meal and the evening meal. Thus it is necessary that those responsible for his welfare during these hours make some plan for his lunch. The child may: (1) eat his meal at home; (2) bring his lunch from home to eat at school; (3) eat lunch in a public eating place; or (4) eat a lunch provided by the school. Any of these may have a place for some child if the meal is served under desirable conditions and provides its share of the day's nutrients at a price the child can afford to pay. The shift in emphasis from the first three to the last has been concomitant with the development of knowledge about the relationship of nutrition to the growth of the child and with changes in patterns of family, school and community living.

Lunch at Home

Going home for lunch has become impractical for many children for a number of reasons, such as the increase in the number of working mothers, the shortening of lunch periods, and the consolidation of schools with resulting greater distances between home and school. Where circumstances permit, lunch at home may be desirable.

The Home-Packed Lunch

The home-packed lunch is still an important item in the nutrition of many children and may be an acceptable way of providing the noon meal. Small schools with limited funds, equipment, and space may be wise to concentrate their efforts on improvement of the home-packed lunch and to supplement it with milk or other foods at school, rather than to open a school lunchroom.

The Public Eating Place

In some areas the public eating place plays an important part in providing the lunch for school children. This is especially true in secondary schools where independence of choice is highly prized. I was told in one community that for high school students to spend 75 cents or one dollar or even more to eat in public eating places is a mark of prestige for them and their parents. This is perhaps an example of "conspicuous consumption" and of the traditional attitude that spending is a measure of success. Purchase of lunch in a public eating place may be satisfactory for some children.

Lunch at School

A nutritionally adequate lunch may be provided in any of the ways already mentioned as well as in the school lunchroom. However, a value peculiar to participation in the lunch program is use of the lunchroom as a laboratory for the practice of good food habits based on scientific principles taught in the classroom. Most schools have no other provision for this activity. The lunchroom is also an ideal setting for the practical application of accepted social behavior and good citizenship practices. Educators who advocate the participation of all children in the school lunch use its educational function as the primary reason.

OBJECTIVES OF THE SCHOOL LUNCH

A broad viewpoint of the school lunch will include both nutritional and educational objectives, which may be stated in this way: the school lunchroom should provide a nutritionally adequate meal served under sanitary conditions at minimum cost, and it should be utilized as a laboratory where good food practices are established.

Nutritional Adequacy

Nutritional adequacy for lunch at school is generally interpreted to mean that it provides at least one-third of the National Research Council's Recommended Daily Dietary Allowances. Freely translated, this means that lunch for the youngster in the fifth grade should provide about 25 g protein, 0.4. g calcium, 4 mg iron, 1500 IU of vitamin A, 0.4 mg thiamine, 0.6 mg riboflavin, 3 mg niacin, and 25 mg ascorbic acid. The kinds and quantities of food which will supply these nutrients will be discussed later.

No matter how well planned, the lunch will meet the nutritional needs of the child only if eaten; hence, it must be so planned and served that it appeals to the youthful patrons. This

implies foods which are well cooked, palatable, attractive and easy to eat, and served under unhurried conditions in pleasant surroundings. It implies also motivation of the child so that he actually eats the food served.

Sanitary Conditions

Sanitary practices in relation to cleanliness of lunchroom and kitchen, health of employees, handling of perishable foods, dishwashing procedures, and disposal of food waste should meet or exceed local regulations. The lunchroom should serve as a laboratory where children may observe good standards of sanitation.

Importance of Cost

Serving the food at minimum cost is important because of the relationship of cost to the number of children who eat in the lunchroom. The author once observed the effect of an increase in price in a small rural school. At the beginning of the school year when the pupils paid 20 cents for lunch, 98 per cent of them ate in the lunchroom. An increase in price to 25 cents caused a drop in participation to 60 per cent; only after several months did it again rise. The relationship between cost and participation seems to be due to the psychologic effect of getting the most for one's money as well as the ability of the family to pay for the lunch.

Educators agree that the lunchroom should operate on a nonprofit basis and that space, equipment, and salaries should be provided in the same way that they are provided for classroom activities. However, in most lunchrooms the amount paid by the children covers part or all of the salaries of lunchroom personnel, while in some schools janitorial or other services are also charged to the lunchroom.

Educational Values

Emphasis in nutrition education in the early years of elementary school should be on the creation of favorable attitudes toward food and the formation of good food habits. In the upper grades the relationship of proper food to nutrition and health and the acceptance of a variety of foods are stressed. At the high school level, nutrition subject matter is taught as a part of various subjects related to health.

The school lunchroom should be used as a laboratory where children put into practice the concepts taught in the classroom. After children have studied what constitutes a good lunch, why the various foods are included, and how the lunch helps meet their nutritional needs, they eat together and see how the foods are used in a meal. They also practice good social behavior and group conduct, and the art of table conversation.

The school lunch has great potentialities for extending nutrition education into the home and the community. Some schools have printed their lunch menus in the local newspaper with suggestions for the meals eaten at home. This is a way to avoid duplication of foods and to help the parent plan the child's total food intake.

EXTENT OF SCHOOL LUNCH PROGRAMS

It has been estimated that half of the school children in the United States have the opportunity to purchase some kind of lunch at school, although in many cases this means only a sandwich and soft drink or other combination of low nutrient content. Consequently, even though some children eat a good lunch at home or in a public eating place or bring good home-packed lunches it has been estimated that less than half of the school children in the United States consume nutritionally adequate lunches.

However, the number has increased greatly as the result of the National School Lunch Act. During 1946–47, the first year of the program, 45,000 schools participated; in 1955–56 over 56,000 schools received federal support. The number of children participating increased from about six million to more than $10^1/_2$ million. About 1,575,000,000 complete meals were served during 1955–56.

THE NATIONAL SCHOOL LUNCH PROGRAM

Although many programs are financed through local sources, material help for the school lunch has been available from the federal government since 1933 when the Reconstruction Finance Corporation provided loans to

several communities in Missouri to pay labor costs of preparing and serving school lunches. Later, federal funds were used to purchase surplus foods for distribution to school lunchrooms. However, the great growth in number of programs has occurred since passage of the National School Lunch Act, Public Law 396, in June, 1946. This growth and the benefits which have accrued as a result of the Act are tributes to the ingenuity and foresight of those who wrote it.

Under the provisions of the Act the federal government has assisted states to start lunch programs. In the first year of federal assistance under the Act, some funds were appropriated for the purchase of equipment but in subsequent years, most of the money has been spent for food. Help is also provided in the form of technical aids made available to all schools participating in the program.

Objectives

Two objectives are stated in the Act: "To safeguard the health and well-being of the Nation's children, and to encourage the domestic consumption of nutritious agricultural commodities and other food." The educational aspects of the program were not mentioned in the Act because these have traditionally been the prerogatives of state and local government.

One other important objective is implied in the Act, that no child shall be unable to eat the school meal because of lack of money. The Act requires that lunch must be provided without cost for children who are unable to pay and specifically prohibits discrimination against these children, such as requiring them to work for their lunches or to sit at a special table.

Provisions for Financial Support

The Act established the procedures for yearly grants-in-aid to support nonprofit school lunch programs, placing the responsibility for administration in the Department of Agriculture. It provides that most of the money is to be spent for food and that at least 75 per cent of it must be allocated to the states with allocations for individual states based on per capita income and the number of school children. It requires that every federal dollar be matched by three dollars from state and local sources. This matching amount is reduced for states in which the per capita income is lower than the average for the United States.

The money is allocated to schools only as reimbursement for the cost of food for children, although this is interpreted to include transportation, storage, and certain other costs as well as the purchase price of food. The school receives reimbursement at a specific rate not to exceed 9 cents per lunch. During the 1956–57 fiscal year the reimbursement rate, which is set by the individual state, varied from about $2^1/_2$ cents to 9 cents, with an average of $4^1/_2$ cents.

Financial support is also provided to the schools by direct distribution of foods purchased with funds made available under the National School Lunch Act, and price support and surplus removal legislation. The foods purchased with school lunch funds are selected to provide nutrients apt to be lacking in children's diets. Federal funds made possible the distribution of the following foods last year: shell eggs, frozen hamburger, canned and frozen pork, frozen turkeys, lard, dry beans, butter, cheese, cornmeal, wheat flour, nonfat dry milk, cabbage, fresh plums, rice, frozen ground beef, peanut butter, orange juice concentrate, grapefruit sections, canned tomatoes, canned corn, canned green beans, canned peaches, canned plums, and tomato paste. Discriminating use of these foods enabled some schools to charge only 20 cents for a lunch which contained foods that would have cost as much as 30 cents if purchased through regular market sources.

Administration of the Act

Responsibility for administering the program is delegated in each state to the educational agency. This agency decides which schools are eligible to participate, sets the reimbursement rate for each lunch, and handles the funds. State prerogatives in relationship to the educational aspects have already been mentioned.

Standards for the Lunch at School

Schools receiving reimbursement from federal

funds are required to provide a meal which meets certain "minimum nutritional requirements." Although three "types" of lunches were originally defined, this discussion will be concerned only with "Type A" which is now most widely served. This pattern has become a guide for other lunches for children.

Minimum nutritional requirements for the Type A lunch are met by a complete meal which provides from one-third to one-half of the Recommended Daily Dietary Allowances for the child. Amounts of specific nutrients were listed at the beginning of this paper.

To assure the desired nutrient content the Agricultural Marketing Service of the Department of Agriculture has specified that the Type A lunch shall contain at least one-half pint of milk as a beverage; 2 oz of lean, cooked meat, poultry, or fish, or 2 oz of cheese, or one-half cup of cooked dried beans or peas, or 4 tablespoons of peanut butter or one egg; three-fourths cup of raw or cooked vegetables and/or fruit; 2 teaspoons of butter or margarine; and at least one slice of whole grain or enriched bread or its equivalent in hot breads.

An example of a well-planned plate menu based on this pattern might include: meat loaf, mashed potatoes, cole slaw, hot biscuits with butter and honey, apricots, and milk. However, requirements may be met by either hot or cold foods, and some schools have successfully served Type A sack lunches and Type A salad lunches. The sack lunch has proved especially popular in spring weather when children like to eat out of doors. A sample menu might include chopped liver sandwich, cole slaw, applesauce, oatmeal cookie, and milk. The salad lunch meets with approval of teenage girls as well as teachers, and might consist of a chef's salad (greens with cheese and meat), hot roll, ice cream, and milk.

COMMUNITY AND SCHOOL RESPONSIBILITY

Many persons consider that the school lunch and even the entire school program are the concern only of parents. However, children develop in a single educational setting comprising home, school, and community, and the kind of citizen produced will determine the characteristics of the community a few years later. Actually, all voters and taxpayers have prerogatives and responsibilities in relation to educational programs, including that for the school lunch. They must be made to realize that the school budget should cover the lunch program as well as other school departments.

A brief review of the roles played by some of the persons concerned will serve as a preface to a few suggestions for specific ways in which the physician may support the school lunch program. Some school districts have school nurses, health coordinators, curriculum supervisors, nutritionists, and others who play a vital role. This discussion will cover only persons found in all schools which serve lunch.

The School Board: This body is responsible for the development of overall policies which determine how the school is operated, financial support, and a determination of the place of the lunchroom in the activities of the school. Members of the Board are subject to pressures from all directions, which may result in an anomaly such as purchase of food for the lunchroom from local merchants at retail prices rather than from wholesale sources, although this results in the necessity of charging pupils a higher price for their lunches. However, most school board members try to look realistically upon their function. The board must delegate responsibility for the school lunch program to the school administrator if it is to be a part of the educational program.

The School Administrator: His is the responsibility of carrying out the policies of the school board. The emphasis placed on the school health program will often be determined at this level. The administrator can provide in-service training or other help for teachers who have limited knowledge of basic nutrition, and he can suggest methods of integrating it into classroom teaching. He is usually responsible for lunchroom personnel and for use of the lunchroom as an educational laboratory.

The Classroom Teacher: She is at the "grass roots level" in the development of good food habits. She is the one who must discover the types of activity which promote learning in nutrition and guide their application in the classroom so that the child changes his prac-

tices. She is usually responsible also for supervision of the lunch hour.

The Lunchroom Manager: She must maintain standards to make the lunchroom a suitable setting for use as a laboratory. She should be trained in lunchroom methods as well as in food service and should have status and salary equivalent to that of a teacher. She must be able to train staff in high standards of food service and to work with teachers and other school personnel to develop lunchroom-centered educational activities.

SOME PROBLEMS OF THE SCHOOL LUNCH

There has been and still exists in some areas opposition or apathy toward school lunch programs and failure to recognize their significance in building community health. It should be remembered that the school lunch is one of the health and welfare programs which have developed rapidly during the last 25 years. These programs have developed as the result of political and social struggle and it seems probable that some of the opposition and apathy have resulted from this struggle. However, those who accept the responsibility for the health of the nation's children must not let these political implications obstruct their progress toward the goal of better health.

Only an analysis of the entire lunch program in a particular school can show its special problems. A few of the most frequently occurring ones will be discussed briefly.

Food Service Rather Than Education

Perhaps the greatest problem and the basis for most of the others is failure to make the lunchroom a part of the educational program and, indeed, failure to include any nutrition education in the school activities. The reason most often heard is that "there isn't time." However, it seems probable that the actual reason lies in the lack of training in nutrition education on the part of administrators, teachers, and lunchroom managers so that no one really knows how to accomplish this goal. Few teachers have had courses in nutrition although some states now require one as part of the work for the elementary teaching credential. Teachers must also be convinced of the value of the school lunch and of the influence of their support on participation by the children.

Indifferent Attitudes of Parents

Sometimes parents feel that because the child will have a good lunch at school his morning and evening meals are of less significance. However, the child who eats lunch at school still gets most of his dietary intake at home. Thus it is vitally important that the parent understand the place of each meal in the total food for the day. In most schools the lunch should aim to provide a third of the day's nutrients, rather than to compensate for the inadequacies of meals served at home.

Unsatisfactory Meals

Many lunches eaten by children fail to provide the recommended amount of nutrients. In some cases this is because *a la carte* service is provided, with the result that an inadequate combination of foods is selected.

Another problem is the service of Type A lunches or other complete meals which do not meet the standards for nutrient content. This is usually due to lack of understanding of the food requirements on the part of an untrained manager or to indifference about their importance. Sometimes, a delicious, attractive meal is low in food value, like one which the author recalls, which featured chicken and noodles with a gelatin dessert. Calculation of the food value showed that the meal was low in almost all nutrients.

Even when an adequate plate lunch is provided, children may leave part of their food with resulting nutritional inadequacy and the tendency to fill up on snacks. This may be due to poorly cooked food or unattractive service but is often because the children have been used to lunches of hamburgers or hot dogs with potato chips, spaghetti or other starchy foods, and do not accept the idea of a complete meal at lunch time. Frequently, they are copying the food patterns of parents or teachers.

Sale of Soft Drinks and Other Snacks

In many schools soft drinks and other snacks are sold in the lunchroom or elsewhere on the

school premises. They compete for the child's appetite as well as his money. A study in one school showed that children from low-income families were spending as much as 65 cents for snacks during the day. Other studies have shown that children selected and ate better lunches whem competition from these items was removed. The American Medical Association, the American Dental Association, the National Congress of Parents and Teachers, and the American Dietetic Association are all on record as disapproving the sale of candy and soft drinks in schools.*

SOME WAYS FOR THE PHYSICIAN TO SUPPORT THE SCHOOL LUNCH

The school's need for the interest and support of all voters and taxpayers has been discussed. Physicians are, however, best able to appreciate the importance of school lunch and other school health programs to the health of children. A physician may, through the local medical association, initiate or cooperate in the formation of a school lunch committee with representatives from each of the health disciplines as well as students, parents, and teachers. When the school staff includes a physician, dentist or dental hygienist, nurse, and nutritionist, the medical group can support their efforts; in other schools, where one or all are lacking, arrangements for representation can be made through the cooperation of appropriate professional organizations and school authorities. The local public health officer can help to plan the membership as well as to interpret the need for the committee and to develop its plan of action. If the services of a nutritionist are not available through the school, a physician may be instrumental in obtaining help through local or state health departments, Agricultural Extension, or the local dietetic association.

The function of this committee is to consider all school lunch matters and to establish policies to govern its operation. It is important that policies and actions be sound, conservative ones, that they represent the group thinking and have the support of all members so that splits over controversial issues are avoided. Although group action is often a slow way to initiate a program, it is, in the long run, the surest way to progress.

Energetic leadership by physicians and other professional persons who recognize the importance of a good lunch to a growing child can guide the thinking of others concerned with the school lunch. This will help to avoid such reports as those recently received by the author, of two schools in which service of a complete meal was discontinued in favor of a sandwich-soup-snack kind of service. In both cases the reason appears to be the acceptance by children, parents, and teachers, of a luncheon pattern of soup and pie, or sandwich and soft drink, rather than recognition of the values of a meal of higher nutrient content.

The medical association may also initiate action for an appraisal of the school lunch program to determine how well it is meeting its educational and nutritional objectives. This kind of study will uncover problems of the type discussed in the last section and provide a specific basis for improvement.

Another nutritional problem in which most schools need professional guidance is the sale of soft drinks and snacks at school. A brief review of the experience of one school may emphasize the importance that policies and action result from careful consideration by a group which represents students and parents as well as the various health disciplines.

Some parents in this school, concerned about the incidence of tooth decay in their children, consulted a local dentist who suggested that one means of attacking this problem was to reduce the use of soft drinks. A few enthusiastic mothers persuaded the parent-teacher group to recommend to the school board that sale of soft drinks at school be discontinued. The school board acceded to the request and banned the sale of soft drinks at school. This set in motion a whole chain of events, including a clamor by the children to be permitted to leave the school grounds during the lunch hour, objections from some parents who saw "no harm in a child's having a bottle of pop," and pres-

* Bibliography at the end of this article includes periodicals of the American Medical Association and Martin's book in which are discussed the actions of all four groups.

sure on the school board from the soft drink vendor who had lost a lucrative account. Soon the administrator, yielding to requests of the children, recommended that the sale of soft drinks be resumed and the request was granted by the school board. If this kind of problem had been considered by a committee which included wide representation, action more acceptable to all concerned might have been initiated.

Physicians as individuals or through groups perform a significant service when they interpret to the community in their personal and professional contacts the objectives and significance of the school lunch. The physician can make his child-patients and their parents aware of the values of the Type A lunch, and help them see the benefits derived from eating lunch at school. Good nutrition habits learned at school can carry over into the home to the advantage of other members of the family.

Perhaps one of the most important things the physician can do is to build prestige for the program. Probably the most powerful influence for getting the teenage boy to eat an adequate lunch, to drink milk and eat green vegetables is for him to know that the elders whom he likes and respects—his doctor, dentist, teacher, coach, and the man next door, as well as his father—feel that these are important things for him to do. Patronage of the lunchroom by the most popular students will be a decisive factor in getting other students to eat there.

The prestige of the lunchroom will also be enhanced by interpretation of its use as an educational setting. Dishwashing in the lunchroom may be either an opportunity for all children to help in and to observe one of the processes by which health is protected, or it may degenerate into routine work by which children from low income families earn their lunches. The physician may help to interpret the importance of good sanitation practices in the lunchroom, and, as in the case of other things learned at school these may carry over into home and community.

SUMMARY

Although there has been a great increase in the number of school lunch programs in the last years, it is probable that less than half of the nation's school children regularly eat adequate lunches. A good meal may be provided in other ways but the school lunch offers opportunities to teach the principles of nutrition, to learn acceptance of a good lunch, and to acquire a taste for many foods. It also provides a channel for the improvement of family food habits.

Facilities for the school lunch should be provided on the same basis as facilities for other educational programs. The price children pay for lunch should cover only the cost of the food with funds for labor provided from other sources. Financial support for some schools may be obtained from the Federal School Lunch Program which is administered in each state by the state educational agency.

The major objectives of the school lunch are to serve a nutritionally adequate meal and to provide a setting for nutrition education. These objectives can best be achieved when those immediately responsible for the lunch have the support and help of community leaders.

Physicians, nurses, dietitians, and other professional persons can play a key role in interpretation of the objectives of the school lunch and in analysis of its needs and solution of its problems. Support may be provided through individual contacts and through activities in professional and civic groups.

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Diet Lists

FROM TIME TO TIME we will publish diet lists which seem to have clinical usefulness. Minor variations exist in all standard diet lists; nevertheless these are representative and may be considered useful guides. Publication does not necessarily imply complete agreement with all details.

The following diet lists may be obtained from Eli Lilly and Company, Indianapolis 6, Indiana. Requests for copies and other inquiries should be addressed to the Medical Services Department of that company.

Moderately Low Sodium Diet

(*Approximately 1.5 g Sodium or 3.75 g Sodium Chloride*)

SUGGESTED MENU PLAN

Breakfast

1 serving fruit
1/2 cup unsalted cooked cereal
1 egg prepared any style without salt
2 slices whole-wheat toast
1 teaspoonful butter or oleomargarine
1 glass milk
Sugar, jelly
Coffee

Lunch

2 1/2 oz meat, unsalted
1/2 cup potato or potato substitute
1/2 cup cooked vegetable
1 serving fruit
2 slices whole-wheat bread
1 teaspoonful butter
1 glass milk
Sugar, jelly
Coffee or tea

Dinner

1 glass fruit juice
2 1/2 oz meat, unsalted
1/2 cup potato or potato substitute
1 serving vegetable salad, with saltless dressing
2 slices whole-wheat bread
1 teaspoonful butter
1 serving fruit
1 glass milk
Sugar, jelly
Coffee or tea

Drink 10 to 15 glasses of water each day.

FOODS ON DIET (*do not add salt in preparation*)

Bread

Six slices of whole-wheat bread or 4 slices of white bread daily. If salt-free bread is eaten, 1/4 teaspoonful of salt may be added to food for seasoning.

One teaspoonful of tomato catchup or prepared mustard may be substituted for 1 slice of whole-wheat bread.

Cereals

All cooked cereals, except "quick-cooking" types, prepared without salt. Shredded wheat, puffed wheat, or puffed rice may be used.

Dairy Products

1. Three glasses of milk daily used as a beverage or in cooking. One serving of vanilla ice cream may be substituted for 1 glass of milk.

2. One egg daily, cooked without salt. (One ounce of fresh meat may be substituted for 1 egg.)

3. Three teaspoonfuls of butter or oleomargarine daily.

4. One-half cup coffee cream daily.

Fruits

One serving (1/2 cup) three times daily of any fruit—fresh, frozen, or canned (unless sodium benzoate has been added as a preservative).

Read labels and avoid those fruits to which a sodium compound has been added. Serve a citrus fruit once daily.

Meats

Two and one-half ounces twice daily.

Fresh lamb, veal, beef, pork, chicken (white meat only), turkey (white meat only), or fresh fish. Bake, broil, or fry in unsalted vegetable shortening. *Use no salt.* Onions, garlic, herbs, paprika, and pepper may be used for flavoring.

Do not use batters, bread crumbs, or sauces.

Potato or Potato Substitutes

One-half cup per serving twice daily.

White potato, sweet potato, macaroni, rice, or spaghetti may be used.

Fresh tomatoes, peppers, or onions may be added to give variety. Do not use salt, cream, or cheese sauces.

Other Vegetables

One-half cup per serving twice daily.

Asparagus, canned beets, broccoli, Brussels sprouts, cabbage, cauliflower, corn, cucumbers, carrots, eggplant, green beans, parsnips, mushrooms or squash, lettuce, Lima beans, okra, onions, peas, red or green peppers, and tomatoes.

Vegetables should be fresh or frozen, unless otherwise indicated. *Cook without salt.* Vegetables may be cooked, or served as salad. Any salt-free dressing may be used.

FOODS TO AVOID

(Read carefully)

Canned foods

1. Vegetables—unless canned without salt.

2. Meats—except special low-sodium canned meats.

3. Fish—except when labeled low in sodium (A low-sodium tuna fish is available).

4. Soups or bouillon cubes.

—— smoked, pickled, or salt-preserved or salt-processed meats and fish, such as luncheon meats, bacon, ham, etc.

—— cheeses, except salt-free cottage cheese.

—— foods prepared with baking powder, baking soda, or prepared "mixes" (such as those for cookies, cakes, crackers, puddings, waffles, etc.).

—— highly salted foods, such as pretzels, peanut butter, potato chips, popcorn, salted nuts, etc.

—— salt-containing condiments and sauces.

1. Vegetable salts—garlic, salt, and onion salt.

2. Prepared sauces—catchup, horse-radish, mustard, steak sauce, etc.

3. Bacon or ham fat or other fat to which salt has been added.

4. *Salt substitutes containing potassium if resin is being taken.*

—— medication not prescribed by your doctor.

Saline laxatives, antacids, and pain or fever-relieving drugs frequently contain large amounts of sodium.

—— chemically softened water for cooking or drinking.

LOW-SODIUM FOODS

(May be taken as directed by the physician)

Beverages

Coca-Cola, cocoa (except Dutch-processed), coffee, fruit juices (except tomato), ginger ale, and tea.

Condiments

Allspice, caraway seeds, cinnamon, curry powder, garlic, mace, mint leaves, mustard powder, nutmeg, onions, paprika, pepper, peppermint extract, parsley, sage, thyme, turmeric, vanilla extract, and vinegar may be used for flavoring.

Fats and Oils

Beef drippings, unsalted butter, olive oil, salad oil, and vegetable shortening (such as Crisco and Spry) may be used.

Miscellaneous

Dry coconut, honey, jam, jelly, marmalade, unsalted peanuts, unsalted walnuts, sugar, pure sugar candy (life savers, lemon drops, rock candy, and peppermints).

These low-sodium foods may be used, as directed, to add flavor, variety, and calories to the diet.

The use of a pressure cooker preserves the natural flavors of meats and vegetables.

USEFUL RECIPES

Salt-Free Bread

1 cake yeast
1/2 tablespoonful sugar
1/2 cup milk
2 tablespoonfuls salt-free vegetable shortening
3 1/2 cups flour (approximately)
1/2 cup water

Crumble yeast and mix with sugar. Heat water, milk, and shortening together in saucepan. Add this to yeast and sugar. Mix well. Add half the flour and mix thoroughly; then add the remaining flour and mix well to make a stiff dough. Knead until smooth and place dough in greased bowl. Spread about 1 teaspoonful of melted shortening over dough. Cover dough with towel and allow to set in warm place until it has raised to double its size. Knead again and shape into loaf. Place in well-greased pan, cover, and let raise until double in bulk. Bake in a moderately hot oven (350°F.).

Cut into 20 slices. One slice contains about 3.5 mg sodium and 97 calories.

Salt-Free Butter

Beat chilled cream with rotary egg beater or electric mixer until butter is formed. Pour off remaining liquid. Wash butter with water. Form and chill.

Your Health Depends upon Your Diet

Eat Only the Quantities of Foods Allowed on the Diet

Eat Only Those Foods on the Approved List

Nutrition News

Fourth Annual Nutrition Conference

The Fourth Annual Nutrition Conference, sponsored by Wayne State University College of Medicine, will be held on Thursday evening and Friday, April 24 and 25, 1958. Speakers on the general subject "Proteins and Amino Acids in Nutrition," will include Drs. F. E. Deatherage, Ohio State University; C. A. Alvehjam, University of Wisconsin; F. E. Rice, Research Laboratories, Swift and Company; and N. S. Scrimshaw, Pan American Sanitary Bureau (Guatemala).

1958 Gordon Research Conference on Food and Nutrition

The Gordon Research Conference for 1958 will be held from June 9 to August 29 at Colby Junior College, New London; New Hampton School, New Hampton; and Kimball Union Academy, Meriden, all in New Hampshire. The program for the section on Food and Nutrition (August 11 to 15, 1958) is as follows:

The Intestinal Absorption of Amino Acids; Aline U. Orton

Amino Acid Balance and Imbalance; Alfred E. Harper

Vegetable Proteins in Human Nutrition; James M. Hundley

Physiological Aspects of Hunger and Satiety; Theodore Van Itallie

Further Observations in Fatty Acid Nutrition; Roselyn Alfin-Slater

Changes in Gross Body Composition and in Adipose Tissue as a Result of Caloric Restriction; Albert Behnke

Hormonal Relationships to Nutrition in Domestic Animals; F. N. Andrews

Hormonal and Nutritional Factors in Osteoporosis; Franklin C. McLean

Food Supplementation in Relation to World Food Shortage; R. R. Williams

Food Supplementation; O. L. Kline

Is Toxicology Enough for a Food Protection Program?; Olaf Mickelson

Nutritional Experiments as an Instrument of Research in Congenital Malformations; Josef Warkany

Recent Developments in Studies of the Relations Between Nutrition and Behavior; Josef Brožek

The Establishment of Nutritional Requirements of Man; Mark Hegsted

Infant Nutritional Requirements, Fact, Fancy and Calculations; Calvin Woodruff

Nutritional Surveys in the Near and Far East; Arnold Schaefer

The Program in Nutrition of the World Health Organization; William J. Darby

Effect of Gibberellin on Plant Growth and Composition; S. H. Wittwer

Effects of Fertilizers on the Nutritional Value of Plants; Kenneth Beeson

Requests for attendance at the Conference, or for any additional information should be addressed to W. George Parks, Director, Department of Chemistry, University of Rhode Island, Kingston, Rhode Island.

A Public Health Approach to Nutrition Problems

The College of Home Economics, Syracuse University, and the Nutrition Bureau, New York State Department of Health, are jointly sponsoring the 12th annual Community Nutrition Institute at Syracuse University to be held June 16–20, 1958.

Among the subjects to be presented are: water and electrolyte metabolism with its implication for health and disease; our knowledge of nutrition in surgery; nutrition in pregnancy; nutrition of the infant and child; intermediary metabolism as it affects mental development; problems of weight control programs.

Further information is obtainable from Dr. Anne Bourquin, College of Home Economics, Syracuse University, Syracuse 10, New York.

World Congress of Gastroenterology

The World Congress of Gastroenterology announces the scientific program for its first World Congress to be held in Washington, D. C., May 25–31, 1958. The main Symposia for the Congress are to be on Peptic Ulcer, Intestinal Infections and Infestations, Malabsorp-

tion, Gastric Carcinoma, and Nutrition and Its Effect on the Liver and Pancreas. The speakers at this Symposium will be: C. H. Best (Canada), J. Caroli (France), C. Davidson (U. S. A.), J. Davies (East Africa), H. Ducci (Chile), N. Scrimshaw (Guatemala), B. Sepulveda and S. Zubiran (Mexico), S. Sherlock (Great Britain), J. Waterlow (British West Indies), and I. Wood (Australia). In addition, over 150 papers have been accepted by the Program Committee for presentation. The Proceedings will be interpreted simultaneously in four languages.

Information pertaining to the Congress is obtainable from Dr. H. Marvin Pollard, Secretary-General, World Congress of Gastroenterology, University Hospital, Ann Arbor, Michigan.

Coming in the May-June Issue:

Symposium on The Mode of Action of Lipotropic Factors in Nutrition

Nutrition Quotes

Food Technology Threat to Good Nutrition

"It would be rash, indeed, to make predictions of what new discoveries science may bring to nutrition in the next five or ten years. My concern is that we are not applying the things that are already known.

"I think that the vitamin craze is just about over, and that new emphasis will be put on proteins and on minerals. It is not yet clear that all benefits of proteins derive simply from the amino acid components, but possibly another five years will clear up this important point. The idea of balance in the diet, which is an old one, will receive more and more attention.

"In Food Technology there have been amazing changes in recent years and we can expect many more. Most of these provide foods in some more convenient form and this is likely to be the dominating consideration rather than good nutrition. In fact, there is a constant and continuing threat to good nutrition in many technological developments, but I find it encouraging that many food companies as well as many people are conscious of this threat. The problem lies in trying to get the information we need to answer the questions raised by each technological change. These developments in technology all cost money and the cost of food must therefore tend upwards."

—L. B. Pett, M.D., *Canadian Services M. J.* p. 199, March 1956.

Nutrition of the Future

"The nutrition of the future will deal with many substances which we do not regard as nutrients today. They are cell nutrients, substances which under normal conditions may be elaborated within the body. We know little about them now. We shall learn much more from tissue culture studies with synthetic media. Many of our hormones, perhaps all of them belong to this category, but they are only a few of the substances which are concerned with cellular metabolism. To introduce complex substances within the cell may be difficult and perhaps impossible, but it may not be necessary to do this. A simple prosthetic group may be all that is needed, a 'stress vitamin.' Perhaps the premature infant dies because he has not learned how to make certain enzymes that his more mature brother can make without difficulty. Perhaps he needs a special set of vitamins to supply the prosthetic groups for these enzymes."

—L. E. Holt, Jr., *Arch. Dis. Childhood* 31: 427, 1956.

Undernutrition and Carbohydrate Metabolism

"At present, the alterations in carbohydrate metabolism due to undernutrition can be most economically explained by the assumption that they consist of (a) a slight decrease in peripheral utilization of glucose, the mechanism of which change is not known, (b) an enhanced effectiveness of insulin in promoting utilization of glucose by the tissues, which also awaits further elucidation, and (c) an enzymatic defect in the liver, which possibly involves phosphorylase and/or phosphoglucomutase."

—N. S. Halmi and B. N. Spirtos. *Am. J. Physiol.* 187: 432, 1956.

The Ubiquity of Nutrition

". . . it is immediately evident that such subjects as biochemistry, bacteriology, pharmacology, and physiology—to mention a few—all have important nutritional aspects. For example, nutrition and biochemistry are so intimately related that it is frequently difficult to distinguish one from the other. The nutritional characteristics of bacteria are of enormous interest to microbiologists; nutritional disorders form a significant fraction of the subject matter of pathology; pharmacology textbooks contain extensive discussions about the use of vitamins, other nutrients, and antinutrients in therapeutics. Recital of the many relationships between nutrition and the basic medical sciences could be an endless affair."

—T. B. Van Itallie and F. J. Stare, *J. Dent. Education* 20: 292, 1956.

The Road Ahead

"But the future of nutrition as I see it, the big future which is just opening up, is the field of nutrition in disease rather than in health. In the last analysis cells which sicken and die from any pathological process do so because they are improperly nourished. They may die because of failure of some one of the 30 odd essential nutrients that we now recognize. They may die because of an abnormal requirement of one of our presently known nutrients. They may also die for lack of what I may call a conditional nutrient, a nutrient needed only in a particular pathological state, a stress nutrient if you will."

—L. E. Holt, Jr., *Arch. Dis. Childhood* 31: 427, 1956.

Nutrition Briefs

A COMPARATIVE study of liver function tests shows that the cephalin cholesterol flocculation test is the best single indicator for distinguishing between obstructive and hepatocellular jaundice. Corroborative information may be obtained from the alkaline phosphatase and thymol turbidity tests.

E. Hill and L. Ziene. *Am. J. Clin. Path.* 27: 6, 1957.

A SINGLE, deficient diet, constantly administered, has been demonstrated to produce a cyclic susceptibility change. This cycle is composed of a primary susceptibility increase, a secondary resistance increase, and lastly, another susceptibility increase. The resistance changes are roughly correlated to periods of depot fat utilization (increased susceptibility), reserve protein utilization (decreased susceptibility), and tissue breakdown subsequent to protein starvation (increased susceptibility).

D. H. Sprunt and C. C. Flanigan. *J. Exper. Med.* 104: 687, 1956.

IN THE CASE of 100 asthmatics, the authors emphasize allergic hypersensitivity to insects which attack the wheat grain, particularly to the excrement of the weevil. The subjects lived in grain-growing regions, especially close to barns and granaries.

R. Voukassovitch and I. Spoujitch. *Sem. Hop. Paris* 31: 1907, 1955.

THE CORRELATION between food dislike and allergic reaction to the disliked food is quite low even among very young children.

M. S. Boyden and J. Glaser. *Ann. Allergy* 15: 277, 1951.

DAILY INUNCTIONS of vitamin A (palmitate) for three to five weeks failed to produce any increase in thickness in the epidermis of healthy human volunteers.

L. Fisher and F. Herrmann. *A. M. A. Arch. Derm.* 75:667, 1957.

IN RIBOFLAVIN deficiency carbohydrated absorption is normal but its utilization is impaired. There are blood and tissue changes in the levels of glucous, glycogen, and lactic acid.

M. R. Nelson, L. Arnrich, and A. F. Morgan. *Fed. Proc.* 16: 394, 1957.

THE DAILY individual intake and expenditure of calories has been compared in 66 subjects divided into four widely different groups. Young and middle-aged women had a mean daily energy expenditure of 2,100 to 2,300 cal; male office clerks, 2,800 military cadets, 3,400; and coal miners, 3,700. In each of the groups there was no significant relationship between caloric expenditure and caloric intake on the same, subsequent, or second day following. No pattern in fluctuations about the mean intake was found during the 7 to 14-day-study period. These findings do not fit the theory stipulating that the regulation of food intake depends on a fairly rapid reaction to a chemical stimulus in the blood. This important subject is presented as an abstract and the full details of the study will be of considerable interest.

J. V. G. A. Durnis. *J. Physiol.* 136: 34P, 1957.

ALTHOUGH total plasma cholesterol level of hens did not vary with age, in males the levels did rise, especially in the second year of life.

H. S. Weiss. *Proc. Soc. Exper. Biol. & Med.* 95: 487, 1957.

STUDIES in the monkey indicate that the citrovorum factor content of the liver reflects both the ascorbic and folic acids intake.

K. P. Misra, C. W. Woodruff, and W. J. Darby. *Fed. Proc.* 16: 394, 1957.

INTRAVENOUSLY administered fat particles become coated with proteins as physiologically occurring chylomicra.

H. C. Meng, J. A. Young, H. H. Shoulders, Jr., and J. B. Youmans. *Fed. Proc.* 16: 392, 1957.

A MAGNESIUM deficiency developed when rats were placed on an atherogenic diet which led to the development of hypocholesteremia with extensive sudanophilia of the heart and aorta.

J. J. Vitale, P. L. White, M. Nakameura, and D. M. Hegsted. *Fed. Proc.* 16: 400, 1957.

DIETARY cobalt apparently lowers the plasma cholesterol concentration and decreases atheromatosis in experimental animals by interfering with the absorption of dietary cholesterol.

D. M. Tennent, C. W. Mushett, G. W. Kuron, W. H. Ott, and H. Siegel. *Fed. Proc.* 16: 399, 1957.

Reviews of Recent Books

Diabète sucré, Diabète insipide, Régulation électrolytique. Rapports de la IV^e^ réunion des endocrinologistes de langue française, ed. by Prof. A. Souleirac. *Annales d'Endocrinologie*, G. Doin et Cie, Editeurs, 1957, and Masson & Cie Editeurs, Paris, pp. 420, 3,800 Francs.

Many branches of scientific research came to a stop in Western Europe in 1939. After the end of the war, for many years, the shortage of financial resources, equipment, and buildings was such that research started again only very slowly. An indirect but unfortunate result was that a new generation of scientists, particularly in rapidly growing sciences such as endocrinology, grew up in this country with no inclination and little capacity for reading the foreign literature. While no self-respecting scientist actually dared express this view aloud in an academic environment, many behaved as though to say that if an article were not in English, it could not be worth reading.

Such prejudices among endocrinologists should be dispelled by the perusal of any recent issues of the *Annales d'Endocrinologie*. They could be shattered equally well by the impressive volume on "Sugar Diabetes, Diabetes Insipidus and Electrolyte Regulation" which embodies lectures given at the 4th Congress of French-speaking Endocrinologists. The symposium comprised three main sessions. Accordingly, the first part of the book is on the role of the adrenal cortex in the regulation of mineral metabolism and comprises three chapters: biochemistry of aldosterone (M. F. Jayle and E. E. Baulieu, Faculty of Medicine, Paris); physiology of aldosterone and clinical observations (A. F. Muller and R. S. Mach, University Clinic, Geneva); physiologic effects of mineralocorticoids (F. Morel, Nuclear Studies Center, Saclay). The second part deals with neurosecretory process and diabetes insipidus. More specifically, it has chapters on recent clinical contributions to the problem of diabetes insipidus (P. A. Bastenie, R. Bellens, V. Conard, and J. C. Demanet, Saint Pierre Hospital, Brussels), on histophysiology of neurosecretory processes (R. Collin and J. Barry, Faculty of Medicine, Nancy), on the biochemistry and biology of neuropituitary hormones (F. Stutinsky). The last part, on the endocrine pancreas and diabetes, was of even greater interest to this reviewer (probably because of his own attention to this subject). The first two chapters, Histopathology of the Islets of Langerhans and Pathogenic Concepts on Diabetes, by L. Desclin and W. Gepts (University Hospital, Brussels) and Histophysiology of the Endocrine Pancreas of Mammals by J. Verne (Faculty of Medicine, Paris), which are supplemented by superb color illustrations, constitute one of the best summaries available on the histology of the pancreas. The next two chapters, on Hypoglycemic Sulfamides, are written by two men from the laboratory where their action was first described. L. Hedon (Faculty of Medicine, Montpellier) retraces the first findings and their implications; A. Loubatieres (*ibid.*) discusses the more recent findings and analyzes possible modes of action. C. de Duve and J. Berthet (University of Louvain) have, in their chapter on Glucagon, given what is by far the most thorough and the most imaginative review on this fascinating "new" hormone. In the last chapter, J. P. Hoet, R. de Meyer, and P. D. Ricci (*ibid.*) give a concise but stimulating review of hormonal regulations of the activity of the endocrine pancreas.

It should be superfluous to say that this book (which is remarkably well edited by Prof. Souleirac and superbly printed) should be read by all persons active in the field of endocrinology. It is perhaps not unnecessary to add that some of the compilation of facts and presentation of ideas printed here (to give but one example, the discussion on the possible roles of glucagon) are simply not available in this form anywhere else.

JEAN MAYER

Aids to Materia Medica and Therapeutics, 4th edition, by J. W. Hadgraft. Bailliere, Tindall and Cox, London (Williams & Wilkins, Baltimore), 1957, pp. 259, $3.25.

The first edition of this little student aid-book appeared 25 years ago. The continued popularity indicates it successfully fills a widespread need. As a pocket reference to established drugs, it presents essential information in an extremely condensed form. Because it is written for the British medical student the terminology of the British Pharmacopoeia is used; nevertheless, it can be equally useful to American readers. It is somewhat puzzling that some products have trade names along with the generic names and others do not. When trade names are given only one or two are listed, which tends to lend unwarranted recommendation to one or the other manufacturer. As a quick reference handbook in which the dose and action are summarized in a few sentences the book serves its purpose well. It is a cousin to a dictionary but no relative to a pharmaceutical textbook. S. O. W.

The Story of Peptic Ulcer. Richard D. Tonkin, M.D., F.R.C.P. W. B. Saunders, Philadelphia, 1957, pp. 71, $2.25.

This is a charming and witty little volume, imaginatively and humorously illustrated by Raymond Keith Hellier, intended to inform and reassure patients with a peptic ulcer.

No one who has been entertained by reading it can pick a serious quarrel with this book or with its admonitions to the ulcer patient to take small, frequent feedings and to cultivate a tranquil state of mind. Since Dr. Tonkin evidently designed it to save himself time explaining the nature of peptic ulcer to his patients, it was probably designed to fill a specific need. However, this reviewer believes that it comes a little short of the mark in telling the ulcer patient what he should know about his disease. For instance, there is no mention made of the use of medication in the treatment program. Although the author creates an atmosphere of confidence and encourages squarely facing the realities of the problem, he does not mention the complications or surgical treatment of peptic ulcer. On the other hand, his treatment of information on the incidence and etiology of peptic ulcer seems a bit confusing and possibly not of intrinsic interest to the ulcer patient. However, the book justifies its own existence by being enjoyable reading for doctor and patient alike. J. B. HAMMOND

A History of Nutrition, by E. V. McCollum, Houghton Mifflin Company, Boston, 1957, pp. 451, $6.00.

There has long been a need for a good history of nutrition. Now a distinguished investigator has undertaken this difficult task. It is to Professor McCollum's credit that he has done an admirable and in many ways unique job.

The subtitle, "The sequence of ideas of nutrition investigations," explains this unusual book. Each chapter concentrates on one aspect of nutrition and carries it from early times to the early 1940's. Vitamins and minerals are covered more thoroughly than are protein, fat, and carbohydrate. Studies on caloric needs are rather lightly passed over. Some of the newest developments (e.g., vitamin B_{12}) are not covered and in view of the rapidly changing picture this may well be left for future historians.

Throughout, the emphasis is on animal experimentation, reflecting the author's own interests. Particularly valuable are the numerous references to pioneering studies hitherto buried in the great mass of "literature." The author and subject indexes are quite helpful. Present and future students of nutrition will find much of value in this well written book. All who work in this field will owe a debt of gratitude to the author for his painstaking labors and even the casual reader will appreciate the tremendous amount of work that has gone into the development of our present day knowledge of nutrition and metabolism. S. O. W.

Dangerous Properties of Industrial Materials, by N. Irving Sax. Reinhold, New York, 1957, pp. 1,221, $22.50.

The forerunner of this book has been the bible for reference in our industrial, medical safety, and industrial hygiene departments for several years. This new edition is more complete and better organized in the sense that there is easy reference to the multiplicity of agents and compounds now prevalent in a chemical industry. The subject matter is deftly handled in that instructions and materials are comprehensively covered in an understandable manner.

Whereas the new edition adds bulk to an already large volume, the new material is particularly apropos and concise. This volume admirably fulfills its purpose, that of a complete, quick reference, for prompt and proper understanding of a diversified number of chemical products.

The brief but complete medical explanation section is particularly welcome. The book will be useful for the industrial physician, the hygienist, and the safety engineer. J. D. PIERCE

Books received for review by THE AMERICAN JOURNAL of CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

The Relation of Psychiatry to Pharmacology by Abraham Wikler, Williams and Wilkins, Baltimore, 1957, pp. 322, $4.00.

Practical Clinical Chemistry by Alma Hiller, Thomas, Springfield, Ill., 1957, pp. 265, $6.50.

How to Eat Well and Live Longer by Leon Patrick, Exposition Press, New York, 1958, pp. 121, $2.75.

Office Gastroenterology by Albert F. R. Andresen, Saunders, Philadelphia, 1958, pp. 707, $14.00.

Drugs—Their Nature, Action and Use by Harry Beckman, Saunders, Philadelphia, 1958, pp. 728, $15.00.

Folk Medicine by D. C. Jarvis, Henry Holt, New York, 1958, pp. 182, $2.95.

Diabetes as a Way of Life by T. S. Danowski, Coward-McCann, New York, 1958, pp. 177, $3.50.

Heart Disease—Cause, Prevention, and Recovery by Philip S. Chen, The Chemical Elements, South Lancaster, Mass., 1958, pp. 189, $3.00.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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FLUORINE

The evidence supporting the view that the fluoride ion is an important micronutrient in dental health as a cariostatic agent continues to accumulate. Data supporting the need for fluoridination of drinking waters in areas have been reviewed previously in this section; it would appear that the addition of fluorine to water supplies in many metropolitan areas is an accomplished fact.

The Evanston Dental Caries Study. XV. The Caries Experience Rates of Two Groups of Evanston Children after Exposure to Fluoridated Waters. I. N. Hill, J. R. Blayney, and W. Wolf. *J. Dent. Res.* 36: 208, 1957.

The findings are reported for Evanston of the dental examinations of six to eight-year-old children who had been exposed to fluoridated water for 71 to 82 months and for 12 to 14-year-old children who had been exposed to fluoridated water for 83 to 94 months. In the younger age group, there was a reduction of approximately 58 per cent in the dental caries experience among the permanent teeth. Among the deciduous teeth of this age group, the reduction was of borderline statistical significance. Among the 12 to 14-year-old children, there was approximately a 22 per cent reduction in dental caries experience rates for the permanent teeth. It was interesting to notice that the children who were six and seven years old in Evanston at the time of this examination had a lower dental experience rate than the children of the same age in Aurora, Ill., who had had a lifetime exposure to water that naturally contained 1.2 ppm of fluoride. As would be anticipated by reason of the number of permanent teeth that were fully developed or already in the mouth at the beginning of fluoridation, the Evanston dental caries rate for the 12 to 14-year-old children was still somewhat above the incidence for the same age group in Aurora. It was postulated that an additional three or four years of fluoridation would be necessary before comparable results were obtained in the 12 to 14-year-old group. Again the remarkable similarity between the results in Evanston and those obtained in other fluoridation communities and the results from areas where fluorides were naturally present lends additional support to the value of fluoridation in various communities of different racial and geographic circumstances. J. H. SHAW

Fluorine in Teeth of Israeli Children, in Relation to Fluorine in Domestic Waters and to Dental Caries Prevalence. J. Gdalia and J. Yardeni. *J. Dent. Res.* 36: 203, 1957.

Fluoride determinations were made on different types of deciduous teeth from 114 children who resided in places where the water supply contained less than 0.3 ppm of fluoride, between 0.5 and 0.6 ppm and between 0.6 and 1.3 ppm. No attempt was made to separate the enamel and dentin prior to the fluoride determinations. Since the reported fluoride levels were on composite samples, they are difficult to relate to the fluoride values that have been reported for enamel and dentin from other areas. However, it is noteworthy that again the relationships between increased fluoride in the drinking water, increased fluoride in the inorganic matter of teeth and decreased dental caries incidence were observed. In many cases strong variations were observed in the fluoride content of teeth from children in the same region and teeth of different types from the same individual. The authors commented upon this fact with some surprise but did not point out that possibly this may have been to a large extent due to different proportions of enamel and dentin in the respective samples.
J. H. SHAW

The State of Fluoride in Drinking Water. I. Feldman, D. Morken, and H. C. Hodge. *J. Dent. Res.* 36: 192, 1957.

These authors point out that the widespread use of sodium silicofluoride for the fluoridation of drinking water has made it important to determine the state of the fluoride in such water. A series of fine equations and calculations were presented to indicate the relative state of the fluoride after equilibrium has been reached under various circumstances with respect to the concentration of other ions in the water. When sodium silicofluoride has dissolved in water, hydrolysis occurs and the fluoride exists in any or all of the following states $SiF_6^=$, SiF_4, F^-, and HF. Knowing the hydrolysis constants of $SiF_6^=$ and SiF_4 and the dissociation constant of HF, the distribution of fluoride in these states may be calculated from the pH and the initial silicofluoride concentration. The hydrolysis constants of $SiF_6^=$ and SiF_4 determined from pH determinations on silicofluoride solutions were found to be 7×10^{-32} and 7×10^{-26} at 29° C. In a drinking water supply with a pH of 5 or higher that has been fluoridated with sodium silicofluoride to the extent of 16 ppm of fluoride or less, it was concluded that all of the silicofluoride is completely hydrolyzed to silicic acid, fluoride ion, and hydrogen fluoride. HF constitutes only a very small fraction of the total fluoride in the water; when the water is ingested and absorbed into the blood stream, all of the fluoride is in the form of fluoride ions.

Examples were given of the influence of other ions on the state of fluoride. When the total content of fluoride was 1 ppm, the percentage of fluoride bound to calcium ranges from about 0.03 per cent for very soft water to about 2.8 per cent for very hard water. The magnesium-bound fluoride ranges from 0.3 per cent for very soft water to 28 per cent for very hard water. In terms of specific examples, when the pH of the water was five and the total calcium content was 1 ppm and the total magnesium was 1 ppm 1.5 per cent of the fluoride existed as HF, 98.2 per cent as F^-, 0.03 per cent as CaF^+, and 0.3 per cent as MgF^+. When the water supply had a pH of five and a total calcium content of 100 ppm, and a total magnesium content of 100 ppm, 1.5 per cent of the total fluoride existed as HF, 67.7 as F^-, 2.8 per cent as CaF^+, and 28 per cent as MgF^+. J. H. SHAW

Experimental Cariostasis in Rats on a Tea Diet of High Fluorine Content. J. F. McClendon and J. Gershon-Cohen. *J. Dent. Res.* 36: 220, 1957.

These authors have long been interested in dietary sources of fluoride, other than water. In the present experiment, studies were conducted to determine whether high-fluoride materials such as instant tea powder and a combination of tea and camellia leaves would alter caries incidence in rats. When instant tea was used in the diet for 40 days at a level of 20 per cent, a dietary fluoride level of 35 ppm was attained. Among the animals that received the instant tea supplement, no carious lesions were found whereas the animals on the same diet without the tea had an average of 1.5 carious lesions. The average fluoride content of the femurs in the control group was 0.017 per cent in comparison with 0.17 per cent for those on the tea diet. In another experiment, instant tea was used at a level of 5 per cent, along with ground camellia leaves at a level of 5 per cent. Under these circumstances, the dietary intake of fluorine was 110 ppm during the 112-day experimental period. The animals on the control diet had an average of 4.6 carious teeth while those on the tea-camellia diet had an average of 1.5 carious teeth. These data indicate that the fluorides present in dietary materials other than water are readily available to the rat and are capable of reducing the incidence of carious lesions. This does not come as new information as far as foods in general are concerned, as the fluorides in food sources other than tea and camellia leaves have been shown to be available and to reduce the dental caries incidence. J. H. SHAW

The presence of fluoride in dietary sources referred to above and the availability of fluoride in many dentifrices plus fluoridination of drinking water may lead to chronic fluorosis as suggested in the following report. The data, in the form presented, are entirely inconclusive. Further investigation of the problem is warranted.

Incipient Fluorine Intoxication from Drinking Water. G. L. Waldbott. *Acta. Med. Scandinav.* 156: 157, 1956.

A clinical syndrome is described consisting of stiffness and pain in the back, pains and paresthesias in arms and legs, gastritis, stomatitis, malaise, visual disturbance, brittle nails in patients consuming drinking water containing fluoride in concentrations around 1 ppm. In 52 patients with the syndrome of "incipient" fluorosis, gastrointestinal and neurologic symptoms predominated. The evidence that these patients had fluorosis is presumptive; the resemblance of the symptoms to those seen in industrial fluoride poisoning and in chronic experimental fluorosis, and the relationship between the ingestion of fluoride water and the symptoms are regarded as important evidence favoring this interpretation. The use of nonfluorine-containing waters for drinking and cooking eliminated all symptoms.

While the report suggests an interesting possibility of symptoms attributable to fluoridination of public drinking water now widely employed as a health measure to reduce dental caries, the evidence supporting this view is not adequate and further studies are required. C. R. SHUMAN

The inclusion of fluoride in dentifrices appears to provide a cariostatic action. Whether this effect is due to local changes in pH, change in bacterial flora, or incorporation of fluoride in the structure of teeth is not clearly defined. Apparently, the ingestion of fluoride is significant from this source.

Urinary Fluorine Levels in Children Following the Clinical Use of a Stannous Fluoride-Containing Dentifrice. R. A. Schweinsberger and J. C. Muhler. *J. Dent. Res.* 35: 760, 1956.

Schweinsberger and Muhler have studied the rate of urinary fluoride excretion in 80 children who were using a stannous fluoride dentifrice, that contained 1,000 ppm fluoride. Urinary fluorides were also determined for 74 children using a nonfluoride dentifrice. Both groups of children resided in Bloomington, Indiana, where the communal water supply contained 0.1 ppm fluorides. Urinary fluoride excretion was also determined for 57 children in Ellettsville, Indiana, where the water contained 0.85 ppm fluorides. The average daily amount of fluoride excreted by the latter group was 0.841 mg. This rate of excretion is highly comparable to data that are available from similar water-fluoride areas. The children in the first and second groups excreted a daily average of 0.218 and 0.220 g of fluoride. Obviously there is no significant increase in fluoride excretion among the children using the stannous fluoride dentifrice. This finding can be interpreted logically to indicate that no significant fluoride ingestion occurs because of tooth brushing with the stannous fluoride dentifrice. The actual concentration of fluoride in the urines of the group of children with stannous fluoride dentifrice was about 15 per cent higher than that of the children with a control dentifrice. However, the amount of urine excreted by the former group was about 15 per cent lower than for the latter. These differences cancel out so that the only important factor for consideration, the absolute fluoride values, is essentially the same for the two groups. These data appear to provide a substantial answer to the frequent criticism that fluoride dentifrices containing sufficiently high fluoride levels that toxicity problems might arise because of the reported swallowing of more than optimal amounts of fluoride during and after toothbrushing. J. H. SHAW

Caries-inhibiting Value of a Dentifrice Containing Stannous Fluoride: First Year Report of a Supervised Toothbrushing Study. W. A. Jordan and J. K. Peterson. *J. Am. Dent. A.* 54: 589, 1957.

Five hundred and five children in the fourth and fifth grades of two elementary schools comprised the control and experimental groups used for conducting a study to determine the effectiveness of a stannous fluoride dentifrice in the control of tooth decay. The children in one school were given a dentifrice that contained 0.4 per cent stannous fluoride and 1 per cent stannous pyrophosphate and had a pH of 4.9. The children in the other school were supplied with an identical dentifrice except that the stannous fluoride and stannous pyrophosphate had been omitted with the resultant increased pH of 6.8. Neither the children, their parents and teachers, nor the examining dentists knew which school received the stannous fluoride-containing dentifrice. After one year, the children who had been in the experimental school had about 35 per cent fewer new decayed, missing, and filled (DMF) teeth than did the children in the control school. The greatest reduction occurred on the buccolingual surfaces (41 per cent) and the least on the occlusal surfaces (23 per cent). Among the children using the stannous fluoride dentifrice, 59 per cent did not develop any new DMF teeth during the survey year in contrast to 44 per cent among the children using the dentifrice without fluoride. No significant increase in the amount of fluoride excreted in the urine occurred among the boys using the fluoride dentifrice.

J. H. SHAW

Important data concerning the metabolism and distribution of fluoride are found in the following reports.

Effect of Various Concentrations of Sodium Fluoride on Parasitic and Free-living Protozoa and Rotifera. W. W. Wantland. *J. Dent. Res.* 35: 763, 1956.

The influence of varied concentrations of sodium fluoride on free-living *Paramecia, Euglena* and *Rotifera* was studied to determine the toxicity for these organisms and to explore the possibility of using one or more of these unicells as a test organism for a biologic assay to predict water fluoride concentration. At levels from 2 to 1,000 ppm (parts per million) of sodium fluoride, *Paramecia* and *Euglena* lived, reproduced, and showed a degree of activity comparable to that in nonfluoride cultures. At a concentration of 1,250 ppm both species lived for 15 days; at 1,400 ppm for eight days and only at 1,500 ppm and higher was life shortened to seven hours or less. The *Rotifera* were somewhat more variable in response and lived for four or five hours to nine days in concentrations of 1,250 to 1,450 ppm of sodium fluoride. Levels of 100 to 300 ppm of sodium fluoride were sufficiently toxic to cause an inactivation of trichomonads that were isolated from the cecum of the hamster. This inactivation could be partially overcome by removal of the sodium fluoride solution and replacement with physiologic saline. At higher levels of fluoride, the inactivation was not reversible.

In another part of the study, attempts were made to develop a bioassay for fluoride concentration in drinking water. First these unicells had to be made less resistant to fluoride. This was done by adding an acid zirconium oxychloride solution to a mixture of the water sample and a mixed culture of *Paramecia* and *Rotifera*. Under these circumstances, water samples with 10 ppm or less of sodium fluoride caused death of the *Paramecia* but not of the *Rotifera;* 20 ppm or more of sodium fluoride caused death of the two species. It is rather difficult to conceive circumstances whereby this would prove to be a particularly useful test. If it could be refined enough to be applied accurately to predict the difference between water samples containing two and three ppm sodium fluoride, limited usefulness might be attained. J. H. SHAW

Distribution in the Soft Tissue of the Rat of Radioactive Fluoride Administered as Sodium Fluoride. J. W. Hein, J. F. Bonner, F. Brudevold, F. A. Smith, and H. C. Hodge. *Nature* 178: 1295, 1956.

Because analysis of soft tissues for fluoride is technically difficult there has been relatively little work on the subject.

In the experiments reported here 1 to 4 ml of sodium fluoride solution containing 1 ppm fluoride and carrying fluorine-18 was given orally or intravenously to three rats. Radioactive fluorine decays rapidly (half life: 115 min) so that there was very little time either for equilibration or preparation of tissue for counting. The activity in the various soft tissues is presented in tabular form. In the case of oral ingestion more than half the dose was still in the gut 30 min afterwards. In those given fluoride by vein the thyroid, kidney, and adrenals contained the greatest concentrations. There was a marked decrease in tissue concentrations between 10 and 90 min after fluoride administration. The work confirms previous findings that fluoride is excreted rapidly, largely by the kidneys, but also in the saliva.

F. E. Hytten

Effect of Sodium Fluoride Administration on Body Changes in Old Rats. W. F. Ramseyer, C. A. H. Smith, and C. M. McCay. *J. Gerontol.* 12: 14, 1957.

In caries-resistant albino rats, the effects of lifelong feeding of low levels of sodium fluoride on teeth, bones, thyroids, and kidneys were studied. Four different doses of the mineral were administered in drinking water. In aged control rats, which had not received the fluoride supplement, all teeth were present and the dental tissues were healthy. However, at 520 days of age some of the experimental rats, especially those which had received larger doses of fluoride, showed caries, loss of teeth and periodontal disease. Fluoride had accumulated in the femurs in direct proportion to the amount ingested, females storing more than males. The thyroids were not affected; the kidneys, however, showed tubular hyperplasia and hypertrophy.

M. Silberberg

BIOCHEMICAL METHODS AND TESTS

New technics for the determination of concentrations of enzymes and substrates in tissues and body fluids are emerging continuously. Recent developments in the measurement of calcium involve the use of a photometric method, flame photometry, and titration with versenate. One advantage of the latter is the ability to determine magnesium from an aliquot.

New Dye Method for Direct Photometric Determination of Calcium. G. R. Kingsley and O. Robnett. *Amer. J. Clin. Path.* 27: 223, 1957.

A determination of calcium is reported in which a purple dye, disodium-1-hydroxy-4-chloro-2,2-diazobenzene-1,8-hydroxy-naphthalene-3,6-disulfonic acid, turns reds in the presence of calcium. Magnesium present does not interfere if the reaction takes place in a strong alkaline solution.

A number of sera, urines, and spinal fluids were assayed for calcium by this method and simultaneously by the flame photometer and a permanganate photoelectric procedure. There was a difference between the methods of only a fraction of a milligram to 2 mg. Slightly lower values for calcium were obtained by the flame photometric methods; urinary and spinal fluid calcium was not satisfactory with a Coleman flame photometer, but was readily determined with the dye method. However, a slight modification of the dye method was required if done on sera containing great amounts of lipids; prior extraction with ether was necessary, or else low calcium values would be obtained by the dye method. No one can evaluate a clinical laboratory method by reading a paper. The reliability of the dye method for calcium determination will be judged by those laboratories that may use it in the future.

E. Cohen

Improved Method for Determination of Calcium and Magnesium in Biologic Fluids by EDTA Titration. M. H. Carr and H. A. Frank. *Am. J. Clin. Pathol.* 26: 1157, 1956.

There is a need for simple, rapid, and accurate methods of analysis of calcium and magnesium in blood and other body fluids. Aside from use of flame photometry, a most recent procedure is the titration of calcium and magnesium with a chelating agent. The method described here represents an improvement over that of any one of a number of others already in the literature. Calcium in the specimen is precipitated as calcium oxalate; the precipitate is washed with dilute ammonium hydroxide, and dissolved in a perchloric acid solution. It is then titrated with disodium ethylenediamine tetraacetate (EDTA), eriochrome black T (EBT) is the indicator of the end point (blue with a greenish tinge). Magnesium can be titrated in an aliquot of supernatant collected early during the analysis; the end point is like that for the calcium determination except that yellow pigment in serum tends to increase the green tinge.

The method, as described, was used for almost two years. Comparisons were made with the other methods from which essential features were adapted for use in the proposed method. Results were presented for serum, urine, spinal fluid, gastric aspiration fluid, and bile. Statistical analyses of the results suggested a greater accuracy of the new modified procedure. Special conditions for the method are the exclusive use of Pyrex glassware, reagent and dilution preparation with freshly distilled, or deconized water, the use of polyethylene storage containers. However, the presence of ferric or ferrous iron would make the detection of the end point impractical, if not impossible for magnesium titration. The use of calcium oxalate as a precipitant is not new and can still be criticized as empirical and dependent on

pH conditions; this the authors admit. In addition, two to four hours may be required for complete precipitation of the calcium. Nevertheless, the new modified procedure is remarkable in its simplicity and its reproducibility. It appears practical for use in the routine clinical laboratory as well as in the nutritional research laboratory. E. COHEN

Tests for fat absorption have been devised which permit assessment of pancreatic and small bowel function. I^{131}-labeled triolein and oleic acid are being widely used as test reagents. The following method permits analysis of clearing of the serum as well as absorption.

Simplified Fat Absorption. K. L. Osmon, W. J. Zinn, and G. K. Wharton. *J.A.M.A.* 164: 663, 1957.

The authors devised a simplified test for fat absorption, which is based on the determination of the turbidity of undiluted venous serum. A photometer is set to read zero against a saline blank, and the turbidity of the undiluted venous blood is read directly from the scale. The ingestion of 1 g of fat per kilogram body weight led to a rise in total lipids which correlated well with the serum turbidity. The total lipid-level rise resulted principally from elevation of neutral fat. In amounts of 0.5 g of fat/kg body weight, the serum turbidity followed the chylomicron values. When this amount of fat was given to patients with pancreatic insufficiency, there was no consistent or significant rise in turbidity, chylomicron count, or total lipid. This test measures two factors: the rate of absorption of lipids from the gastrointestinal tract and the rate of clearing of serum after ingestion of the test material. Further work evaluating this simplified test clearly is necessary. S. O. WAIFE

Radioactive vitamin B_{12} absorption tests have become the most reliable method for diagnosis of pernicious anemia. The direct measurement of the radioactivity of blood following ingestion of the labeled vitamin may provide a more useful procedure than the urinary excretion test.

Radioactive Vitamin B_{12} Absorption Studies: Results of Direct Measurement of Radioactivity in the Blood. A. Doscherholmen and P. S. Hagen. *Blood* 12: 336, 1957.

This interesting article reports on further studies into the pathogenesis of megaloblastic anemias utilizing radioactive vitamin B_{12}. Instead of relying upon indirect measurements of the absorption of Co^{60}-vitamin B_{12}, such as in the urine or feces, direct measurements of the radioactivity of blood following a physiologic (0.46 to 0.92 μg) oral dose were performed. Because of the long half-life of Co^{60}, only small amounts of radioactivity may be given, thus necessitating counting procedures especially adapted for low-activity work. Twenty ml of blood was counted in a scintillation well-type counter, for a total of 12,800 counts. Forty-one such tests were carried out in 36 nonpernicious anemia control subjects and 17 tests on nine patients with pernicious anemia.

There was clear separation, although small, of the control and pernicious anemia patients. In addition, the addition of intrinsic factor concentrate resulted in a significant increase in the radioactivity of the blood. It was interesting that four of the nine patients with pernicious anemia did exhibit significant, although less than control, radioactivity in the blood after the oral dose of Co^{60}-labeled vitamin B_{12} even without added intrinsic factor. This reaffirms the fact that patients with pernicious anemia may absorb deficient amounts of food B_{12}.

On serial determinations of the blood content the peak activity was reached 8 to 12 hours after the oral dose. This suggests to the author that vitamin B_{12} may be trapped by the mucosal cells of the intestine. An alternative explanation could be that vitamin B_{12} is primarily absorbed via the portal system, stored in liver and only slowly released into the blood.

There is much to be said for this method in the diagnosis of pernicious anemia. It is easy to perform, requires less cooperation from the patient, does not interfere with bone marrow morphology and bypasses the problem of the effect of disordered renal function on the widely used Schilling Test. The attainment of higher activity with shorter half-life cobalt may make this procedure even more useful. J. F. MUELLER

Radioactive Vitamin B_{12} Studies. Experience with the Urinary Excretion Test and the Measurement of Absorbed Plasma Radioactivity. S. R. Goldberg, B. K. Trivedi, and L. Oliner. *J. Lab. & Clin. Med.* 49: 583, 1957.

The authors present a comparison of the results using the urinary excretion of radioactive vitamin B_{12} labeled with $cobalt^{60}$ versus the plasma levels following the oral administration of $cobalt^{58}$ vitamin B_{12} in patients with macrocytic anemia.

One microcurie was necessary to insure adequate counting levels and a larger dose would be preferable. When $cobalt^{56}$ or $cobalt^{58}$ with shorter half-lives becomes commercially available it will be possible to administer a larger dose. The authors point out that the plasma determinations would be preferable in that urinary collections and the "flushing" dose of stable vitamin B_{12} could be eliminated. There appeared to be a good separation between the healthy volunteers and the patients with pernicious anemia using the plasma levels of radioactivity.

This type of test is a very useful diagnostic precedure and any modification which simplified the procedure will be a welcome addition to the present technic.

K. R. CRISPELL

A Modified Urinary Excretion Test for Measuring Oral $Cobalt^{60}$ Labeled Vitamin B_{12} Absorption and its Application in Certain Diseased States. A. Miller, H. F. Corbus, and J. F. Sullivan. *Blood* 12: 347, 1957.

Amplification of the need for a more direct measure of absorption of orally administered Co^{60} vitamin B_{12}

such as reported above is indicated by this report. Although a modification of the original Schilling Test is accomplished by two parenteral flushing doses of nonradioactive vitamin B_{12} at 3 and 24 hours after the oral dose which results in more recovery of the Co^{60} in normals, it does tend to further complicate the test. In addition, it does not prevent the false positive tests in some patients with acute infections and renal insufficiency. Although the Schilling Test and such modifications as herein reported continue to be of extreme value in the diagnosis of pernicious anemia, it would seem that a test utilizing direct measurement of the blood would be superior. J. F. MUELLER

For many years, a rapid, practical, and accurate method for the determination of blood sugar has been sought. To date, the "perfect" test has not been developed.

A Quick and Easy Method for Blood-Sugar Estimation. M. H. Davies, and R. G. Paley. *Brit. M. J.* 1: 501, 1957.

A kit is available commercially for rapid estimation of blood sugar. One tablet dissolved in a measured amount of water in a test tube precipitates protein in the blood sample which is filtered into another tube. A second tablet is added to the filtrate, the fluid boils, and after 30 seconds gives a color reaction which can be compared to a scale provided.

Blood samples were tested by this technic and by the standard Hagehorn-Jensen method and the results were in remarkable agreement. No sample with an "orange" reaction with the new test had less than 160 mg/100 ml and only one sample with a blue reaction had a sugar content of more than 125 mg/100 ml. Interpretation improves with practice and it is believed that the method would be helpful in medical emergencies.

F. E. HYTTEN

A Rapid Method for the Estimation of Blood Sugar. R. A. Osborn. *Brit. M. J.* 1: 502, 1957.

Blood sugar estimations with a new blood sugar kit (see abstract of *Brit. M. J.* 1: 501, 1957) have been compared with estimations by the method of Kingsley and Reinhold. The correlation coefficient between the two sets of estimations was high (0.84), but there was a tendency for the rapid method to overestimate the blood sugar. The new method has been found useful for the management of diabetic patients in hospitals and is particularly valuable when biochemical services are not readily available. F. E. HYTTEN

A Simple Quick Test for Glucose in Urine. Report on Use of Clinistix. G. Luntz. *Brit. M. J.* 1: 499, 1957.

A rapid method for testing urine for glucose has become available commercially. A piece of thick paper, about the size of a match, and impregnated with reagents containing an enzyme system specific to glucose, is dipped into urine. It turns blue in the presence of glucose. The method has been tried for five months in an outpatient department and has proved to be an accurate and simple screening procedure.

F. E. HYTTEN

Manual of American Society of Clinical Pathologists Workshop on Glucose. F. W. Sunderman, B. E. Copeland, R. P. MacFate, V. E. Martens, H. N. Nauman, and G. F. Stevenson. *Am. J. Clin. Path.* 26: 1355, 1956.

The results of a symposium on the measurement of blood glucose and the identification of reducing sugars in urine are reported.

Methods currently used to determine glucose are dependent upon certain properties of the glucose molecule: (a) reduction powers due to the aldehyde moiety; (b) optical activity that can be measured by the polarimeter; (c) liberation of CO_2 during fermentation by yeasts; (d) susceptibility to oxidation by an enzyme (glucose oxidase).

A combination of the reduction and fermentation methods with measurement of the resultant difference in reducing ability may be the most accurate procedure. It is not suitable, however, for routine clinical use. On the other hand, glucose oxidase, obtained from *Aspergillus niger* and *Penicillium notatum* has been used to analyze glucose by specific catalysis of the conversion of beta glucose to gluconic acid. Reduction methods are most suitable for routine clinical use. Reducing substances other than glucose may give rise to errors of from 5 to 50 mg/100 ml, depending on which reduction method is used. Difficulties in interpretation of "true" blood glucose values frequently are due to a lack of understanding of technical limitations of the method. Another source of error may be glycolysis of the substance, when the oxalated blood specimen is kept at room temperature. Analyses for glucose should be done as soon as possible after collection of the specimen. The concentration of glucose in a protein-free filtrate of blood, however, is stable for forty-eight hours under refrigerator conditions of storage. For oxalated blood, preservation for forty-eight hours under refrigeration, 10 mg of sodium fluoride per ml will prevent glycolysis; 0.5 mg of thymol per ml of blood will prevent fungal contamination. Potassium oxalate 10 to 20 mg per 10 ml of blood is the most common anticoagulant. A suitable mixture of preservative and anticoagulant is composed of 10 parts of powdered sodium fluoride, 1 part of powdered thymol, 3 parts of powdered oxalate; only 4 mg of the mixture is necessary for 1 ml of blood. EDTA has no preservative properties and no advantage over potassium oxalate. The blood proteins that interfere with accurate glucose analysis must be removed. Protein precipitation methods are presented in a detailed summary; actual choice depends on the volume of work. Tungstic acid is the most frequent precipitant.

The Sunderman-Fuller macro method, and the Sunderman micro method are described in detail, with

adaptations for both visual and photoelectric colorimetry. No reason was given for omission of excellent modified Somogyi methods. Technics and examples of distinguishing pentosuria from glycosuria are presented in useful detailed summaries. The precise methodology and calculations of the gravimetric calibration of pipettes are described. E. COHEN

The intravenous glucose tolerance test has not been widely used because of differences in technic and interpretation of results. The technic of this important test should be standardized to provide a more accurate index of glucose tolerance.

The Practical Application of the Rapid Intravenous Glucose Tolerance Test. D. S. Amztuzio, E. D. Rames, and S. Nesbitt. *J. Lab. & Clin. Med.* 48: 714, 1956.

The authors describe a simple and rapid glucose tolerance test using 25 g of glucose given intravenously over a four minute period. The variable factor of absorption from the alimentary tract was thus eliminated.

In a control group of 103 normal men, the difference in glucose concentration compared to the fasting level at 68 minutes during this rapid intravenous glucose tolerance test ranged from −28 to −26 mg/100 ml of glucose. In 20 of these normal men the glucose differences at 68 minutes were almost identical on repetition of the test several days later. Thirty-nine patients with known diabetes mellitus showed a separation from the normal controls with a mean excess of +50.4 mg/100 ml. The range was from +20 to +102 mg, meaning there was a slight overlap with the control group. The intravenous test was normal in all 14 patients with hyperthyroidism who were tested. The oral glucose tolerance in the same group gave abnormal results in 5 of the 14 patients.

Values of glucose excess greater than +26 mg/100 ml at 68 minutes are considered abnormal by the authors and indicate a diminished glucose tolerance.

K. R. CRISPELL

The Interpretation of the Intravenous Glucose Tolerance Test. J. D. Baird and L. J. P. Duncan. *Clin. Sc.* 16: 147, 1957.

Two indices of glucose tolerance have been recently defined. Both are measured as the decrease in blood glucose concentration per minute. However, one, the "total index" (T. I.) is expressed as a percentage of the total blood glucose concentration at that time, and the other, the "increment index" (I. I.) is expressed as a percentage of the increment in concentration above the fasting level. Clearly, both concepts cannot be right.

While in the hospital, 35 mild diabetic patients, 45 to 73 years of age, were treated with carbutamide. No insulin was given. Blood sugar was estimated after fasting and after the two main meals each day. Ten subjects were given an oral glucose tolerance test and eleven an intravenous test. The blood-sugar concentration was considerably lowered by carbutamide treatment, but the daily variation was not altered. For the intravenous glucose tolerance test, the mean increment index for the control period was 1.45 and for the treatment period 1.46; individual variation was very slight. The mean total index rose from 0.50 to 0.77.

Thus while the increment index indicates no change in glucose tolerance, the total index would indicate an improved tolerance under treatment. It is suggested that the increment index is physiologically more logical and is the best available measure of glucose tolerance.

F. E. HYTTEN

Determination of Cocarboxylase in Blood. W. W. Kay and K. C. Murfitt. *Biochem. J.* 62: 601, 1956.

In the method described, cocarboxylase in heated blood cells is recombined with apocarboxylase prepared from yeast, and the resulting haloenzyme is employed in the decarboxylation of pyruvic acid, by a manometric technique for the measurement of evolved CO_2.

Innovations include the use of Norite charcoal to obtain a blank-free apoenzyme, the addition of cyanide to the system (to overcome effects of interfering substances), and the use of an internal standard during assay. The authors point out the effects of adding thiamine and iodoacetate to the system and discuss the possible significance of the sulfhydryl form of cocarboxylase in decarboxylation. M. K. HORWITT

Specific Enzymic Method for the Estimation of L-Tryptophan. L. H. Frank and R. D. DeMoss. *Arch. Biochem.* 67: 387, 1957.

Tryptophanase prepared from *Escherichia coli*, strain Crookes (ATCC No. 8739) is used to convert L-tryptophan to indole which is continuously extracted with toluene, and assayed colorimetrically using *p*-dimethylaminobenzaldehyde reagent. The method is highly specific for samples of L-tryptophan from 0.1 to 1.0 μ moles, and with proper precautions D-tryptophan, indoleacetic acid, 3-hydroxyanthranilic acid, tryptamine, isatin, oxindole, kynurenine, 5-hydroxytryptamine, skatole, anthranilic acid, and casamino acids did not interfere with the assay. Some modifications are necessary in the presence of 5-hydroxytryptophan.

M. K. HORWITT

A Method of Measuring the pH of Body Fluids. V. Wynn and J. Ludbrook. *Lancet* 1: 1068, 1957.

For laboratory workers concerned with the measurement of pH in small blood samples a method and apparatus is described which may be of considerable interest. As little as 0.5 ml of blood can be used and is maintained anaerobically and at controlled temperature. The standard deviation for the reproducibility of pH measurement is 0.005 pH units. F. E. HYTTEN

Modified Turbidimetric Procedure for Determination of Cholesterol. R. L. Costello and G. L. Curran. *Am. J. Clin. Pathol.* 27: 108, 1957.

Until recent years the determination of serum cholesterol and its esters has been time consuming. The authors modified one of the more recent methods, namely that of Pollak and Wadler in which the digitonide of cholesterol is obtained prior to quantitative determination by nephelometry. Unfortunately, the nephelometric end point is inaccurate at the portion of the range where a quantitative precipitation of cholesterol occurs; furthermore, the digitonide is not suspended in a stable manner.

The authors extract serum with a mixture of acetone and ethanol, saponify with potassium hydroxide, precipitate with digitonin, and read the optical density of the suspension in a spectrophotometer. One chief difference from the method of Sperry and Webb is precipitation of the digitonide in the cold for only one hour against 16 to 18 hours by the older procedure. Another difference is the use of a turbidimetric (light-stopping) measurement rather than the nephlos (light-reflecting) type of measurement.

Critical comparisons of the modified procedure were made with a Bloor type technic (Saifer-Kammerer) and the Sperry-Webb method. Higher values were obtained with the modified Pollak-Wadler procedure than with the Sperry-Webb method. Under the conditions of the method and the range of levels of cholesterol studied, the concentration of cholesterol was found to be directly proportional to the optical density of the suspended digitonide.

Research and clinical laboratory workers will no doubt welcome this modified Pollak and Wadler procedure if it proves as rapid, sensitive, yet specific, as the authors claim. E. COHEN

ITEMS OF GENERAL INTEREST

Structure of Bone in Relation to Growth. S. M. Clarke and J. Iboll. *Nature* **179:** 94, 1956.

Femora from rats, hamsters and man were examined by x-ray diffraction at various ages. It was found that the orientation of apatite crystals in relation to collagen fibers changes with age. In man, for example, there appears to be no preferred orientation of the crystals before a fetal age of four months. Thereafter orientation occurs and is marked at birth.

F. E. HYTTEN

Effects of STH on Experimental Lathyrism. H. Selye and P. Bois. *Proc. Soc. Exp. Biol. & Med.* 94: 133, 1957.

Young female rats of the Sprague-Dawley strain weighing 55 g received 5 mg of aminoacetonitrile hydrosulfate plus 1 mg STH for 10 to 29 days. A similar experiment was carried out in a group of rats 100 days old which were treated for periods up to 40 days. STH markedly aggravated the skeletal lesions produced by lathyrism in the long bones and ribs.

M. SILBERBERG

The Occurrence of Multiple Fractures in Suckling Rats Injected with β-Aminopropionitrile (Lathyrism). J. J. Clemmons and D. M. Angevine. *Am. J. Pathol.* 33: 175, 1957.

Suckling rats of the Sprague-Dawley strain received two or three injections of 0.1 cc of a 2 per cent solution of β-aminopropionitrile. The animals were sacrificed on the fourth day and showed osteoporosis and fractures of the long bones associated with degeneration of osteoblasts. The basic change induced is considered to be a defect of the matrix with ensuing inability to calcify.

M. SILBERBERG

Absorption of Topically Applied Vitamins. A. E. Schaefer, H. L. Sassaman, A. Slocum, and R. D. Greene. *J. Nutrition* 59: 171, 1956.

It is well known that in ulcerative colitis, obstructive jaundice, enteritis, and various nonspecific diarrheas and dysenteries, absorption of essential nutrients from the intestinal tract may be impaired. This condition may be associated with a weakening of the metabolic body processes due to the lack of absorption of the essential dietary nutrients. Medical literature contains reports of the failure of patients to respond to either the oral or parenteral administration of nutrients and drugs. On the other hand, there are reports that niacin topically applied to the skin lesions of pellagrins caused a dramatic response in healing of the lesions, and these type of lesions require a long period of oral therapy to produce complete remission. There are also reports in the medical literature that patients with seborrheic dermatitis of the "sicca" type failed to respond to oral or parenteral administration of 300 to 1000 mg per day of pyridoxine for three to four weeks. After discontinuation of parenterally administered pyridoxine this vitamin was applied either in the form of an ointment or as a solution in 20 per cent alcohol, both at a concentration of 10 mg per gram. By this type of therapy the treated lesions cleared within 5 to 21 days, while control areas were unchanged. These results indicate that in certain diseases there may be a metabolic defect in the epidermal cells, so that oral or parenteral administration of the vitamin fails to reach the affected site. Consequently, experiments were conducted to study the absorption of topically applied vitamins by the rat.

In view of the small doses of the vitamins (thiamine, riboflavin, pantothenic acid, pyridoxine, and vitamin D) administered and the limited time allowed for absorption, the utilization of the vitamins studied is considered highly efficient. In addition to the body weight-gain responses, and the prevention or cure of deficiency symptoms, the data on urinary excretion of thiamine and riboflavin substantiate the conclusion. In general, a plasticized hydrocarbon water-absorbing gel ointment base was superior to a carbowax preparation as a vehicle for skin transfer of the vitamins studied. An alcoholic vanillin solution was also an effective vehicle for the skin absorption of riboflavin, pantothenic acid, pyridoxine, and vitamin D. B. SURE

Clinical and Chemical Studies in Human Lactation. IX. Breast Feeding in Hospital. F. E. Hytten. *Brit. M. J.* 2: 1447, 1954.

In the Aberdeen Maternity Hospital it is assumed that breast feeding will be attempted unless there are definite medical contraindications, and 85 per cent of patients leave the hospital fully breast feeding, including 13 per cent who had some initial difficulty. The causes of failure or difficulty in the hospital have been examined in 6,456 married patients who were delivered of single babies which survived the lying-in period. Discharge from the hospital usually took place 10 to 12 days after delivery. Thirty-nine per cent of the failures were considered to be due to inadequacy of lactation, 17 per cent to "ill baby" (usually prematurity) and 15 per cent to maternal unwillingness to nurse. Seventeen per cent were due to anatomically poor, or cracked, nipples. Other less important causes were infant lethargy, maternal disease, and breast engorgement or mastitis. Young mothers were significantly more successful at breast feeding than older mothers. The causes of failure most significantly related to high maternal age were inadequacy of lactation and "maternal opposition." Differences in breast feeding rates at discharge from the hospital were much less significantly associated with maternal age. A. M. THOMSON

Nutrients in Central American Beans. O. B. Tandon, R. Bressani, N. S. Scrimshaw, and F. Le Beau. *J. Agric. Food Chem.* 5: 137, 1957.

It is not unusual for beans to account for 20 to 30 per cent of the protein in the diets of people in Central America. This is a study of factors affecting protein, methionine, lysine, tryptophan, niacin, thiamine, and riboflavin content in 25 bean varieties in two different locations in Gautemala. Differences in composition are reported in detail in relation to variety, location and soil fertility. In general, conditions that increased the content of one nutrient increased also the content of others; in no case was an increase in one correlated with a decrease in another. "The most effective way to increase the amount of these nutrients in beans is selection and distribution of the varieties which are richer in these nutrients." F. E. RICE

Water and Food Intake and the Inhibitory Effect of Amphetamine on Drinking and Eating before and after "Prefrontal Lobotomy" in Dogs. B. Andersson and S. Larsson. *Acta Physiol. Scandinav.* 39: 22, 1956.

Forebrain sections were prepared just in front of the orbital sulcus in dogs. These sections were well anterior to the head of the caudate nucleus. Water intake was not reduced after hypertonic saline by this lesion. When the lesion involved the pre-optic area on both sides, water intake was reduced for a short time after hypertonic saline. Amphetamine blocked water drinking completely after intravenous 20 per cent NaCl. This effect was no longer observed after "prefrontal lobotomy." Incomplete lesions required larger doses of amphetamine to inhibit food intake completely. M. J. OPPENHEIMER

Oral Manifestations of Vitamin Deficiencies. C. A. Medina. *Oral Surg.* 9: 1060, 1956.

This review article briefly describes the nature of the primary nutritional and secondary nutritional deficiencies, and also the oral manifestations of vitamin deficiencies. The description of the individual vitamin deficiencies is terse and by no means complete. In addition the references listed are largely other review articles rather than primary source material. While this review does bring to the dentist a brief bird's-eye view of vitamin deficiencies, it leaves much to be desired. J. H. SHAW

Schonlein-Henoch Syndrome due to Chocolate. J. Richmond and S. Davidson. *Scot. Med. J.* 1: 239, 1956.

The subject is an 11-year-old boy. He was admitted to the hospital with an acute purpural attack, including abdominal pain and hematemesis. While in the hospital he gradually recovered until a fresh attack was precipitated, apparently by a chocolate biscuit. Testing demonstrated that a purpuric attack could be precipitated within a few minutes of giving chocolate either by mouth or by a scratch in the skin. The boy was seen again three months after recovery and was then found to be no longer sensitive to chocolate. Recovery is thought to be due to spontaneous desensitization, although the reason for development of the sensitivity is entirely obscure. F. E. HYTTEN

Idiosyncrasy to Fructose. R. A. Chambers and R. T. C. Pratt. *Lancet* 2: 340, 1956.

A 24-year-old woman is described who had an intolerance to fructose which was first noticed when she was weaned at the age of 10 months. She was the only child of unrelated parents, neither of whom had the intolerance.

On investigation she became nauseated after ingestion of 10 g of fructose and larger amounts led to severe nausea and vomiting. There was no evidence of disordered glucose metabolism.

Idiosyncrasy to fructose may be a rare cause of difficulty in weaning. F. E. HYTTEN

Physiological Role of Antibiotics as Factors in Senescence. C. Calet and R. Jacquot. *Compt. Rend Acad. Sc.* 240: 991, 1955.

The increase of blood lipid levels with no increase in protein, the reduction of energy consumption evidenced by an improved diet caloric turnover, reduction in desoxyribonucleic acid and in the nucleoplasmatic ratio in the liver, lead the authors to postulate that oral Aureomycin acts as a senescent agent rather than a growth factor. H. GOUNELLE